EVALUATION OF THE TOXIC EFFECTS OF ELTENAC (4-[(2,6-dichlorophenyl) amino]-3-thiophene acetic acid), A NONSTEROIDAL ANTI-INFLAMMATORY DRUG, IN HORSES

by

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A TOXICITY STUDY OF ELTENAC (4-[(2,6-dichlorophenyl) amino]-3-thiophene acetic acid), A NOVEL NONSTEROIDAL ANTI-INFLAMMATORY DRUG, IN HORSES

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(ABSTRACT)

A controlled study was performed to determine the potential toxic effects of the new nonsteroidal anti-inflammatory drug, eltenac (4-[(2,6-dichlorophenyl) amino]-3-thiopheneacetic acid), in horses. Four treatment groups were formed, each composed of 6 horses. The drug was injected intravenously, once daily, at a dose rate of 0.5 mg/kg, 1.5mg/kg or 2.5 mg/kg for 15 days. A control group was injected with sterile saline solution. Parameters assessed were hay and water consumption, daily clinical observations (evaluation of attitude, mentation, pulse and respiratory rate, fecal consistency, skin condition, and color and hydration of mucous membranes), physical examinations, complete hemogram, coagulation profiles, serum biochemical profiles, urinalysis, gastroscopic examinations and gross post-mortem and histopathological examinations on all organ

systems. All examiners were blinded to group assignment and dosage levels until the completion of the study. A few glandular gastric ulcers, mild in severity, developed in seven animals during the treatment period. This occurred more often in horses treated with high doses of eltenac (P=.02). A dose dependent change of WBC count and neutrophil count were noted. Total protein, albumin and globulin levels had dose dependent decreases. One horse in the high dose group (2.5mg/kg) developed ventral edema as well as hypoproteinemia. None of the horses in any of the dosage groups exhibited depression or anorexia. Gross post-mortem and histologic examination did not reveal any signs of drug related gastrointestinal, renal or hepatic abnormalities. Minimal toxic effects of eltenac given intravenously were greatest in horses treated with 2.5 mg/kg of the compound for 15 days.

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Jim Terrell

whose friendship, encouragement and unwavering support made this possible.

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The 24 research horses that had to be sacrificed for this study, their lives have contributed to finding a safer nonsteroidal anti-inflammatory drug, for use in the horse.

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LIST OF ABBREVIATIONS

NSAID = Nonsteroidal anti-inflammatory drug
C-13 = Carbon 13
PGE = Prostaglandin E
TXA = Thromboxane A
SRS-A = Slow reacting substance of anaphylaxis
5-HPETE = 5-Hydroxyeicosantrienoic acid
mg = milligrams
kg = kiligrams
g = grams
lb = pounds
dl = decaliter
µl = microliter
ADH = Antidiuretic hormone
cAMP = cyclic adenosine monophosphate
BUN = Blood urea nitrogen
IV = Intravenous
BID = Two times, daily
TID = Three times, daily
PCV = Packed cell volume
CrCl = Chromium chloride
GGT = Gamma glutamyltransferase
WBC = white blood cell
RBC = red blood cell
HGB = hemaglobin
HCT = hematocrit

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAID's) are one of the most commonly used drugs in horses due to their beneficial anti-inflammatory, analgesic and antipyretic effects.[1] By inhibiting cyclooxygenase during the arachidonic acid cascade, NSAID's can induce beneficial as well as toxic effects.[2] Toxic effects associated with NSAID use in horses include gastrointestinal erosion, ulceration, diarrhea, anorexia, depression, hypoproteinemia[3] and renal papillary necrosis.[4, 5]

Mechanisms of NSAID toxicity have been studied in humans[6] and horses[3, 6-10] however, the exact mechanisms contributing to all the adverse reactions to this class of therapeutic agents have not yet been identified. Inhibition of prostaglandin synthesis is reported to interfere with the "cytoprotection" in the gastrointestinal tract.[11] This could contribute to gastrointestinal erosion and ulceration. Other studies suggest that NSAID's cause microvascular necrosis within the gastrointestinal tract as being responsible for NSAID-inducted intestinal injury.[9, 10] The relationship of this mechanism for toxicity to inhibition of cylcooxygenase is unclear.

When phenylbutazone was first used in horses, it was generally considered nontoxic.[12, 13] However, subsequent studies described severe adverse side effects when it was administered to normal horses.[2, 3, 14] Given the unique gastrointestinal and renal toxicity caused by NSAIDs in horses,

toxicity studies have become an integral part of evaluating the NSAIDs before clinical use.

Eltenac, 4-(2,6-dichloroanilino)-3-thiophene acetic acid, is a cylcooxygenase inhibitor. In a previous clinical efficacy study eltenac proved to be an excellent analgesic and anti-inflammatory drug for horses with either post-operative wound swelling or musculoskeletal pain.[15] No adverse reactions were noted during a 7 day period of administration. The purpose of the current study was to determine the potential toxic effects of eltenac when administered intravenously to horses at a dose rate of 0.5mg/kg (anticipated clinical dose), 1.5 mg/kg, and 2.5 mg/kg once daily for 15 days.

LITERATURE REVIEW

Historical Background

The Greeks defined inflammation 2000 years ago as the presence of heat, redness, swelling, pain and loss of function. Since that period mankind has searched to develop a substance that would alleviate those symptoms. Before the Christian era, physicians prepared extracts from the bark of the willow tree (Salix) and used them to treat a wide variety of disorders. It was not until the early 19th century, however, that the active components of willow bark were identified as salicin and salicylic acid, which would later be recognized as the first nonsteroidal anti-inflammatory drug (NSAID). Sodium salicylate was first used in 1875 for the treatment of rheumatic fever in man.[16]

Shortly following the use of sodium salicylate for treatment of disease in humans, Dun, in 1895, reported the successful use of acetylsalicylic acid (aspirin) in horses. He reported that "Mr. E. Price, Birmingham, is satisfied with the effects of horses, and prescribes 10 grains, repeated every two hours, gradually increased to a drachm, and reports the disappearance of the rheumatic pains in 48 hours".[17] Aspirin remained the only NSAID in clinical use until the discovery of phenylbutazone, which was introduced into human medicine around the time of World War II. It was introduced shortly thereafter into veterinary

medicine. Because phenylbutazone has caused deaths in people from aplastic anemia and agranulocytosis, its use in human patients is now restricted.[18] There are also therapeutic limitations for corticosteroid derivatives, which are useful for treatment of inflammatory disorders. Because the pharmaceutical industry recognizes the huge market for a good anti-inflammatory drug, research in the nonsteroidal anti-inflammatory field has been significant in the last 20 years.[18] Flunixin meglumine, meclofenamic acid, naproxen, ibuprofen and ketoprofen are examples of commonly used NSAIDs that have been developed within that time.

Presently, there are numerous reports of clinical efficacy, toxic effects, biochemical and morphologic change induced by NSAIDs in the equine.[5, 7, 8, 12, 18-21] Most recently, new NSAIDs that have been studied for use in the equine market are carprofen,[22-27] eltenac[15] and miloxicam.[28]

Chemical Classification

The nonsteroidal anti-inflammatory drugs all share properties responsible for their anti-inflammatory activity. All are acidic with a pKa of 4.5 or less. The acidic nature of the NSAIDs results in plasma protein binding of 95 to 99 percent.[18]

The two chemical classifications of NSAIDs are the carboxylic acids and the enolic acids (Table 1). Enolic acids exist in two isomeric forms, keto and enol. The hydrogen atom of the carboxylic group or of the enolic isomer reacts with bases to form sodium salts.[29, 30] The common NSAID's phenylbutazone, naproxen, ketoprofen, and flunixin meglumine are discussed in this review (Table 1).

Mechanism of Action of NSAIDs on Inflammation

Part of an inflammatory response results in the production of eicosanoids from the metabolism of long chain polyunsaturated fatty acids, such as arachidonic acid.[31] In mammalian species eicosanoids are composed of prostaglandins, thromboxanes and leukotrienes.

The initiation of eicosanoid synthesis begins with cells releasing arachadonic acid when triggered by trauma, anoxia, endotoxins or products of the host defense response.[32] This mechanism is catalyzed by the enzyme phospholipase A_2. The

activation of phospholipase A_2 has been hypothesized to be the rate limiting step in the synthesis of the eicosanoids.[32, 33] Arachadonic acid is metabolized by two distinct enzymatic pathways; the cyclo-oxygenase pathway and the lipoxygenase pathway.

The cyclooxygenase pathway results in the production of the eicosanoids, prostaglandins and thromboxanes (Figure 1). In 1971, Vane [34] discovered that aspirin and indomethacin inhibited or blocked the cyclooxygenase enzyme and decreased the deleterious effects produced by the prostaglandins and thromboxanes in inflammation. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase by preventing abstraction of hydrogen from C-13 which in turn blocks peroxidation at C-11 or C-15.[35] This abstraction is highly specific, since similar abstraction as well as peroxidation reactions at other points in the fatty acid molecule are not inhibited.[35]

Some reports suggest there are two forms of cyclooxygenase. One, PGHS-1, is expressed in most tissues and is described as a "housekeeping" enzyme regulating normal cellular processes such as maintaining the integrity of the gastric and duodenal mucosa.[36] Another form of the cylooxygenase enzyme is PGHS-2 which is reported to increase during inflammation. DeWitt el al. demonstrated that certain NSAID's selectively inhibit the two forms of cyclooxygenase thereby resulting in less toxic effects of certain NSAIDs.[37]

Prostaglandins and thromboxanes are synthesized in different quantities depending on the cell type that is a participant in inflammation. The endothelial cell is a source of prostaglandin synthesis, specifically PGE_2 and PGI_2.[38] In comparison, the mononuclear phagocytes produce a wide spectrum of prostaglandins (including PGE_2 and PGI_2), thromboxanes, and leukotrienes.[39] In contrast, platelets are a major source of thromboxanes. [39] Polymorphonuclear leukocytes are poor sources of both thromboxanes and prostaglandins. [39] The polymorphonuclear leukocytes are, however, a major source of leukotrienes.[40]

Prostaglandins have many roles in inflammation. A few of the deleterious effects include; hyperalgesia, through interaction with histamine, serotonin and substance P; pyrexia, by effects on the hypothalamus; and edema, caused by increased blood flow and increased vascular permeability.[31] Although prostaglandins are often associated with unwanted effects of inflammation they are important in vascular and cellular homeostasis. These beneficial effects include increased blood flow to the gastric mucosa, abatement of gastric acid production, and the increase of renal blood flow during times of reduced renal perfusion.[41, 42]

Thromboxanes (TXA_2, TXB_2) are produced primarily by platelets. They have powerful vasoconstrictive properties as well as platelet aggregating abilities. The thromboxanes are important in blood clot formation.[41] When arachidonic acid is released from

platelet phospholipids, a potent but unstable platelet aggregating agent and vasoconstrictor, thromboxane A_2 (TXA_2), is produced from cyclic endoperoxides by the action of cyclooxygenase on arachidonic acid. The potent aggregating effect of TXA_2 in platelets is inhibited by the production of prostaglandins E_1 and D_2 (PGE_1 and PGD_2) from linolenic and linoleic acids. [41]

The leukotrienes are the products of the metabolism of arachadonic acid by way of the lipoxygenase pathway. Examples of the leukotrienes are 5HPETE, LTA_4 (previously called SRS-A or slow reacting substance of anaphylaxis), LTB_4, LTC_4. Leukotrienes also have a role as potent mediators of chemotaxis, chemokinesis, aggregation and degranulation of PMN leukocytes as well as PMN leukocyte adherence to endothelial cells. They cause suppression of T-cell mitogenesis with lymphocytes and have potent smooth muscle contractive properties in the bronchi, trachea and pulmonary artery.[43]

Most of the NSAIDs available today inhibit or block the enzyme cylcooxygenase and thus combat the effects produced by the eicosanoids. Certain NSAIDs bind irreversibly to cylooxygenase and others possess a reversible binding capacity. Aspirin, phenylbutazone and meclofenamic acid bind irreversibly to cyclooxygenase while a metabolite of phenylbutazone, oxyphenbutazone, appears to bind reversibly.[2] It is proposed that the difference in binding capacity may be responsible for the discrepancy between short half-life (1.6 and 4.5 hours for

phenylbutazone and flunixin, respectively) and long duration of action (24 - 72 hours for each).[2] Two studies reported that flunixin meglumine and phenylbutazone concentrations in inflammatory exudate were higher than plasma concentrations 12 hours after these drugs were administered.[29, 42] This suggests that the level of NSAID found in plasma may not be indicative of the level found in inflammatory tissues.

The inhibition of cyclooxygenase has been proven to enhance the production of leukotrienes.[44] This was shown by measuring SRS-A (produced by the lipooxygenase pathway) production in three stimulated sections of human lung each treated with various NSAIDs. In this study indomethacin was found to potentiate the production of SRS-A, ketoprofen inhibited production of SRS-A (now known as LTA_4). This suggests that ketoprofen inhibits the lipoxygenase pathway. However, this study was done in human lung sections and the ability of ketoprofen to inhibit lipoxygenase in the horse has not been proven.

An inhibitor of both lipoxygenase and cyclooxygenase would alter the vascular and cellular effects of inflammation similar to the corticosteroids, but without the detrimental effects associated with steroid use. The compound BW755C, a dual lipoxygenase and cylcooxygenase inhibitor, has been studied, [35, 45-47] but to date no drug is available on the market that has this capability.

Pharmacology of NSAIDs

A high proportion of circulating NSAIDs is bound to plasma proteins.[42] Ninety nine percent of the total amount in plasma may be protein bound, which accounts for the relatively low proportions of most NSAIDs in saliva or excreted unchanged in urine.[2]

Relatively little information is available on the metabolism and excretion of the NSAIDs in horses with the exception of phenylbutazone.[1] Phenylbutazone is presumably metabolized in the liver by hydroxylation[2] and two derivatives, oxyphenbutazone and gamma-hydroxyphenylbutazone, have been identified from this process.[48, 49] The active metabolite oxyphenbutazone inhibits further phenylbutazone metabolism and thus increases its half-life. This may explain the dose dependent kinetics reported for phenylbutazone in the horse.[50]

A wide range of plasma half lives have been reported for the NSAIDs in horses. [22, 49, 51] Flunixin meglumine has a short half life of 1.6 hours,[22] naproxen has a plasma half life of 4-5 hours, [49] and phenylbutazone has a half life ranging from 3.5 to 10.9 hours, increasing as the drug dosage increases and with accumulation in the body.[51]

Although the half lives of these drugs are relatively short, studies have demonstrated that when both phenylbutazone and flunixin are given as single, intravenous, therapeutic doses to

ponies, the formation of prostaglandin E_2 in inflammatory exudates is reduced for 12 to 24 hours.[52, 53] This discrepancy between the short half-life and long duration of action of this drug can be partially explained by the observation that many NSAIDs bind irreversibly to cyclooxygenase, requiring the synthesis of new enzymes at the site of inflammation before more eicosanoids can be generated.[2] Another explanation for the discrepancy between half life and duration of action is suggested by the observation that flunixin meglumine and phenylbutazone concentrations in inflammatory exudate were higher than plasma concentrations at 12 hours.[53] Therefore, plasma concentrations may not necessarily correlate to levels in inflamed tissue.[42]

Toxicity of Nonsteroidal Anti-inflammatory Drugs

By inhibiting the cyclooxygenase pathway, NSAIDs provide many beneficial effects such as reduced inflammation, analgesia and reductions of fever. However, along with these benefits come undesirable effects. Toxic effects of the NSAIDs have been well documented in man and laboratory animals for over 30 years.[1] In horses, the toxic effects of a few commonly used NSAIDs have been reported.[3, 5, 14, 19, 42, 54, 55]

The most commonly encountered side-effect in both veterinary and human patients is gastrointestinal irritation and ulceration causing plasma or blood loss into the gut.[2] Early

manifestations of NSAID toxicity in horses include depression, anorexia and a decrease in serum total proteins.[14] More severe adverse effects that have been reported include oral, gastric, duodenal, and colonic ulceration and necrosis, diarrhea, hematological and serum biochemical disturbances, renal papillary necrosis and death.[3, 5, 7-10, 14, 52, 54] In man other toxic effects are observed which include edema, hepatotoxicity, blood dyscrasias and skin rashes.[54, 56]

Gastrointestinal toxicity resulting in ulceration, edema, hypoproteinemia, diarrhea and death have been the most common detrimental effects associated with NSAIDs in horses.[3, 8, 13, 18, 52, 57, 58] This has also been seen in man, rats, dogs, and cats.[59-61] Although the mechanism has not been defined, there is a common belief that gastrointestinal toxicity associated with NSAIDs is caused by their ability to inhibit the cyclooxygenase pathway thus inhibiting prostaglandin synthesis as prostaglandins protect the gastric mucosa.[62, 63]

Pathophysiology of cellular damage in NSAID-induced gastrointestinal ulceration

Mucosal cell injury in the gastrointestinal tract develops through multiple steps and pathways.[63] The following are factors which are important in mucosal cellular injury:

Oxygen is important in cellular injury. Ischemia, or lack or oxygen, and reperfusion can generate the production of free radicals which are chemicals with unpaired electrons. The free radicals usually are the result of incomplete reduction of O_2 to water through the mitochondrial electron transport system.[63] Reports of studies performed in mice have shown that free radicals increase in gastric mucosal injury induced by NSAIDs (aspirin)[64] Other factors that may contribute to cellular injury are; release of intracellular calcium followed by activation of damaging enzymes; decreased ATP production causing loss of ion gradients; and activation of proteases which cause the destruction of intracellular and extracellular structural proteins.[63]

Cell adhesion molecules are also involved in cellular injury during ulcer formation induced by NSAIDs. These molecules on the neutrophils and endothelial cells are activated causing neutrophil adherence to the endothelium. The resultant decreased capillary flow exacerbates ischemic and hypoxic cell injury.[65]

The most immediate repair of damaged epithelium is by rapid restitution. If the mucosal lesion is very superficial and

blood flow is maintained, epithelial cells rapidly respond by expanding pseudopodia and migrate to cover the epithelial defect.[63] Deep mucosal necrosis causes cessation of blood flow in a relatively large area, destroying basement membrane in addition to cell loss[63] resulting in a longer healing period.

Restitution is an inherent property of the GI mucosa. The process of cellular restitution requires energy, and, therefore, microcirculation to the mucosa is crucial for both repair of ulceration and maintenance of the protective mechanisms.[63]

Gastrointestinal prostaglandins and their role in cytoprotection

The intestinal prostaglandins of the E, F, and I types have been found in the gastric mucosa, gastric juice, intestinal mucosa and intestinal secretion of various species.[62, 66-69] Prostaglandins have been associated with relaxation and constriction of the lower esophageal sphincter, contraction of the longitudinal smooth muscle, contraction and relaxation of the circular smooth muscle of the intestine, induction of diarrhea, and the inhibition of small bowel absorption of electrolytes and water.[69] Prostaglandins have been shown to be important in gastric acid secretion. Prostaglandins of the E, A, and I series, for example, are inhibitors of basal and stimulated gastric acid secretion in rats, dogs, cats and humans.[67-69] The administration

of prostaglandins abated ulcer formation in animals receiving ulcerogens such as NSAIDs,[7, 70-73] stress,[74] and oral ethanol, strong acids and bases.[70, 75, 76]

Although the antisecretory properties of prostaglandins have been proven, other mechanisms may inhibit ulceration. One review (protective effects) of prostaglandins on gastric mucosa proposed a four part hypothesis.[62] First, although the various prostaglandins have many effects on blood supply, all provide comparable protection against experimentally induced ulcers. Secondly, when prostaglandins are administered in nonantisecretory doses, protection against gastric ulceration is maintained. Thirdly, other antisecretory agents such as anticholinergics and histamine H2 receptor blockers did not provide full mucosal protection during various experimental ulcerogenic interventions despite the fact that prostaglandins were fully protective under the same conditions.[62] Finally, ulcerations in the intestine, where a nonacidic environment exists, could be prevented by prostaglandins.[77]

The term "cytoprotection" is used to describe the protective effects of prostaglandins.[62, 77] These properties of cytoprotectants include prevention of gastric mucosal barrier disruption, stimulation of mucus secretion, enhancement of gastric mucosal blood flow, stimulation of nonparietal cell alkaline secretion, stimulation of macromolecular synthesis, cellular transport processes and sodium transport and CAMP, stabilization

of tissue lysosomes, dissolution of gastric mucosal folds, maintenance of gastric mucosal sulfhydryl compounds, and stimulation of surface active phospholipids.[78, 79] Based on numerous studies it is concluded that the prostaglandins' have numerous physiologic effects which when combined, provide cytoprotection of the gastrointestinal mucosa. [77] Protection was also observed in ponies where NSAID toxicosis (induced by phenyl-butazone) was inhibited by orally administered prostaglandins (PGE_2).[7]

Some reports have questioned the significance of prostaglandins' activity within the stomach and intestine of the horse.[9, 10] In these studies NSAID toxicity was induced with intravenous phenylbutazone at 13.6 mg/kg daily. Prostacyclin and prostaglandin E_2 levels were then measured in the stomach and intestine after 24, 48, 72 and 96 hours during treatment and compared to pretreatment. No change in prostaglandin concentrations was observed in both the stomach and intestinal mucosa at any sampling time. During the same experiment, diffuse microvascular injury was observed in areas of gastric, duodenal and colonic erosion. These observations agree with other NSAID toxicity studies which reported occlusion of microvascular circulation as a cause of reduced mucosal perfusion.[63, 79] Another study examined the role of adhesion molecules in NSAID induced toxicity.[65] In that study monoclonal

antibodies directed against the adhesion molecules inhibited gastric injury induced by indomethacin.[65]

Adherence of neutrophils to the vascular endothelium can be stimulated by leukotreine B_4. It is felt by some researchers that the inhibition of cyclooxygenase with subsequent leukotriene production stimulates the adhesion of the leukocyte to the endothelium, and that the resultant vascular damage is largely responsible for NSAID induced gastrointestinal ulceration.[79] Prostaglandins have been shown to inhibit neutrophil adherence, chemotaxis, and secretion in response to various agonists, suggesting a potential role in preventing vascular damage.[79] The conclusions from these studies suggests other mechanisms may cause NSAID toxicity rather than a deficiency of prostaglandins in the intestinal mucosa causing loss of cytoprotection.

Phenylbutazone

Phenylbutazone was first synthesized in 1946 and later marketed as Butazolidin (R) by J.R. Geigy. The detrimental effects in man were soon documented.[80] Its toxic effects in the equine, however, were not reported for twenty years.[12] One of the first major studies which reported the first toxic effects of phenylbutazone in the horse was in 1962.[81] In that study phenylbutazone given in single doses at 1-16 grams did not cause any gastrointestinal lesions. Ulcerative lesions were found in the

intestine, stomach and oral mucosa of horses given 8-16 grams of phenylbutazone daily for an undetermined period of time. In 1977, in a review by Gabel [12] the drug was considered safe and "side effects and toxicity were rare at the usual doses". In that paper, the statement was made that "toxicity to phenylbutazone is almost never seen in the equine patients in spite of close observation and periodic blood counts done during thousands of courses of therapy". The safety of phenylbutazone was further discussed in a review in 1977.[13] A study reporting the toxic effects in 1979 phenylbutazone was administered to ponies at 10 g/kg/day (2 times therapeutic dose) for 7-14 days and resulted in ulceration of the oral cavity and stomach, edema of the large colon and cecum, as well as peritonitis.[3] Gastrointestinal effects, specifically the development of submucosal edema, were considered to contribute to the deaths of three ponies. The authors also suggested that phenylbutazone was perhaps more toxic in ponies than horses. In another report phenylbutazone given at 10-12 mg/kg/day for 8-10 days resulted in loss of appetite and massive ulceration of the large colon and cecum.[14] Large accumulations of bacteria were seen in the necrotic mucosa of the intestinal tract. Submucosal edema, vasculitis with perivascular hemorrhage and leukocytic infiltration of vessel walls in the mucosa was also seen. Based on excretion studies using $^{51}CrCl_3$, this study concluded that the hypoproteinemia observed during toxicity occurred due to the lesions seen in the gastrointestinal

tract. A few horses in this study however, exhibited loss of protein in the absence of obvious intestinal lesions.

Foals given phenylbutazone at 10mg/kg/day for 12-42 days exhibited severe gastric and oral ulcerations and edema of the right dorsal colon.[82] At the time of that report (1983) the recommended dose of phenylbutazone was 8.9 mg/kg/day for 3-4 days followed by 4.4 mg/kg/day for 4 days.

Phenylbutazone's toxic effects on the gastrointestinal tract were reported in both horses and ponies in varying doses in further studies.[5, 7, 18, 52] Various doses of phenylbutazone have been recommended since phenylbutazone was first marketed. In a later study in 1983 a revised dosage of phenylbutazone was recommended at 4.4 mg/kg twice daily for 1 day, followed by 2.2 mg/kg twice daily for four days, then 2.2 mg/kg daily for seven days.[83] Although this dose resulted in changes of a few serum biochemical variables, no gastrointestinal effects were seen. This dosage remains the current recommended dosage for phenylbutazone in the horse.

It has been hypothesized that phenylbutazone causes severe gastrointestinal ulceration due to 1) its affinity of phenylbutazone to an acidic environment, 2) the reduction of prostacyclin formation in the stomach leading to increased acid secretion, and 3) local vasoconstriction leading to tissue hypoxia.[51] The first explanation is disputed since toxicity is also seen in the oral cavity, cecum and right dorsal colon.[81, 82] The second and third

explanations are disputed by the studies of Meschter[9, 10] in which phenylbutazone toxicosis was induced and no correlation was found between mucosal prostaglandin concentrations and pyloric erosions. From these studies it was concluded that microvascular damage with focal ischemia caused the initial ulcerations. Although prostaglandin E_2 has exhibited a protective effect on the mucosa during phenylbutazone toxicosis, this study concluded that phenylbutazone may damage cells by mechanisms other than inhibiting prostaglandin production.

In response to research findings, the use of phenylbutazone has drastically changed over the last 20 years. The recommended doses of phenylbutazone have been revised to lessen the gastrointestinal toxicity.[3, 83] Evidence from studies of Collins et al.[7] suggest that phenylbutazone is more toxic in ponies than horses. Furthermore, it has been learned in both experimental toxicity studies and clinical trials that phenylbutazone induced gastric ulcers can be reversed with discontinuation of the drug[7] or lessened with concurrent administration of sucralfate, a mucosal protectant, and ranitidine, a histamine type-2 receptor antagonist.[85, 86]

Flunixin meglumine

Like phenylbutazone, flunixin meglumine is a cyclooxygenase inhibitor and anti-inflammatory agent. It was introduced into veterinary use in the late 1970's. In one study in the administration of 1, 3, and 5 times the recommended intravenous dose (1.1 mg/kg BID) for 10 days did not cause gastrointesinal lesions.[22] However, a case of toxicity was reported in 1984 in which a dose rate of 3 mg/kg of flunixin meglumine was administered twice daily for five days.[87] Gastrointestinal toxicity was manifested as anorexia, depression, and oral ulceration.

Toxicity was again reported in other case reviews[87, 88] and in a safety study of chronic flunixin therapy in foals.[89] In the study on foals, recommended dosages of flunixin meglumine at1.1 mg/day for 30 days, induced oral and gastric ulceration as well as erosions of the glandular stomach mucosa.

Clinical and pathological effects of flunixin meglumine were also evaluated in foals over a period of 5 days at dosages of 0.55 mg/kg, 1.1 mg/kg, 2.2 mg/kg, and 6.6 mg/kg. No clinicopathological effects were noted at recommended dosages, however, at 6.6 mg/kg/day (6 times recommended dosage) gastrointestinal ulceration and cecal petechiation were found.[89]

Flunixin meglumine is less toxic to the gastrointestinal tract than phenylbutazone.[90] When dosages of phenylbutazone (4.4

mg/kg), flunixin meglumine (1.1 mg/kg) and ketoprofen (2.2 mg/kg) were compared via intravenous administration every 8 hours, flunixin was found to be least likely to cause less severe toxic gastrointestinal effects.[90] In that study however, the compounds were given at greater than the recommended dosages and comparisons are difficult because the increased dosages were not proportional among the test compounds. In light of this study, and the study that used flunixin meglumine to successfully induce gastric ulceration in foals,[89] gastrointestinal toxicity is recognized as a possible complication of flunixin meglumine therapy.

Ketoprofen

Ketoprofen was approved for use in horses in 1990. In humans, in addition to blocking the cylcooxygenase pathway, ketoprofen inhibits the lipoxygenase pathway. This mechanism however, was only exhibited in human lung preparations and has not been proven in the horse. [90] The toxic effects of ketoprofen in horses has only been reported in one study.[90] Five of five horses treated developed lesions in the gastric glandular mucosa, however the area of lesion was not found to be significantly different from lesions in saline treated horses. It appears from the study that ketoprofen was the least toxic NSAID when compared to phenylbutazone and flunixin meglumine. The reason for the low toxicity is not known.

Naproxen

Naproxen is a NSAID that also inhibits the cyclo-oxygenase pathway. It is used on a limited basis for myositis and has been reported to induce gastrointestinal toxicosis in dogs.[92] To date no reports of gastrointestinal toxicity in the equine have appeared in the literature. This may be, however, due to its limited use compared to phenylbutazone and flunixin meglumine.

Renal Toxicity

Pathophysiology - Prostaglandins play an important role in the homeostasis and maintenance of kidney function.[92] In the kidney the prostaglandins cause vasodilatation and thus promote increased renal blood flow and glomerular filtration , inhibit sodium retention, and promote free water excretion.[93] Prostaglandins also influence renal regulation of potassium via effects on angiotension release and aldosterone secretion.[93] The exact mechanism has not been defined, but it is suggested that alteration of vasodilitory effects of prostaglandins play a role in NSAID toxicity.[93, 94] Prostaglandins are proposed to maintain kidney function by several different mechanisms.[93] First, prostaglandins tend to enhance renal blood flow and glomerular filtration rate, thus encouraging the delivery of filtrate to the

distal nephron sites. Second, by decreasing resistance in the efferent arterioles, prostaglandins alter peritubular Starling forces thereby decreasing reabsorption of sodium and water, and enhancing delivery to the distal nephron where dilution takes place. Third, by increasing renal blood flow through the medullary region, prostaglandins decrease papillary hypertonicity, which assists in free water excretion. Lastly, prostaglandins may directly interfere with sodium reabsorption in collecting ducts, which further decreases hypertonicity. In addition, prostaglandins inhibit ADH induced cAMP synthesis which interferes with the action of ADH and thus limits water reabsorption in the collecting duct.[95-97]

NSAID toxicity in horses can result in renal papillary necrosis.[5] In man, renal papillary necrosis is a common lesion of analgesic nephropathy,[94] a condition also found in rats.[98] In man, prostaglandins have an important role in regulation of renal blood flow in the case of volume depletion, hemorrhagic hypotension, or anesthesia. In these cases, the renin-angiotension system becomes activated by prostaglandins and blood flow increases. [5, 92] In a review of NSAID induced renal papillary necrosis in horses two main etiologic factors are proposed for the role of NSAIDs.[5, 92] First, renal blood flow becomes dependent upon prostaglandins due to dehydration, hemorrhage, or water deprivation when reduced blood flow stimulates the renin-angiotension system. Secondly, NSAIDs or drugs which inhibit

prostaglandin formation can cause a reduction of the blood supply in the prostaglandin-dependent vasa recta, resulting in ischemia of the renal papillae.

There are very few clinical reports of NSAID induced renal disease occurring in the equine species. In most toxicity studies, renal papillary necrosis accompanies biochemical changes.[3, 99, 100] End stage renal failure, however, has not been reported. In a study on renal papillary necrosis in horses, most of the renal findings were incidental, however, two of 16 horses may have exhibited clinical signs due to NSAID toxicity.[5] In this study, the most common gross lesion was in the kidneys and was described as yellow-green cavitations of papillae in both kidneys, specifically at the poles. Histologically, changes described were as follows: "papillary necrosis with a specific demarcation of viable medulla, deposition of oxalate and calcium phosphate crystals in the necrotic tissue, dilation of collection ducts and Henle's loops and hemorrhage in the viable medulla adjacent to necrotic zone. Variable sized fragments of necrotic papilla often were sequestered into calyces."

NSAID induced renal papillary necrosis in horses, as in man,[100, 101] is thought to be reversible by discontinuation of the drug.

Phenylbutazone

In one of the first toxicity studies in which phenylbutazone toxicity in ponies was recognized, adverse renal effects were not reported.[3] Although many studies had in-depth descriptions of gastrointestinal effects, no renal lesions were reported.[3, 12-14, 19, 81] Finally, a study reported the occurrence of renal papillary necrosis in horses receiving NSAID administration as part of therapy before death.[4] In this paper, 16 horses were examined in which renal papillary necrosis was seen at necropsy. Two horses had shown signs of clinical renal disease. Gunson and Soma reported similar lesions following phenylbutazone administration while concurrently causing a state of dehydration.[5] In this paper, no adverse clinical signs were seen in water deprived horses or when phenylbutazone was administered to the horse at 8.8 mg/kg/day for 4 to 90 days. Horses that were treated with phenylbutazone at 8.8 mg/kg/day phenylbutazone for 4-75 days as well as having water deprivation for 36 to 48 hours prior to euthanasia exhibited gross and histologic renal changes though no clinical signs were evident. Gross changes included yellow-green radial streaks occupying the papillae of both kidneys. Zones of intense hyperemia surrounding these streaks were evident in the medulla. There was sloughing of the renal pelvic epithelium including the lining of the collecting duct, and coagulative necrosis

of the papillary interstitium. Within the calyx, mineralization of necrotic debris was often seen.

In a retrospective study of 269 horses treated with phenylbutazone, 20 horses had signs considered to be evidence of phenylbutazone toxicosis.[8] Of those 20 cases, 2 horses had evidence of renal dysfunction and in the horse that died, papillary necrosis was seen. The horse that lived had isosthenuria in the face of dehydration. Another study of phenylbutazone toxicity, renal lesions were seen in all horses after administration of 15 or 30 mg/kg of phenylbutazone daily for up to two weeks.[19] In this study renal papillary necrosis and medullary interstitial edema were observed. Microscopically, tubular epithelial cell necrosis was seen mainly in the collecting tubules. Changes in serum biochemical parameters included; increased serum concentration BUN, creatinine, and phosphorus and a decrease in serum calcium. These changes were also observed in ponies when doses of 10 mg/kg were administered to 10 ponies once daily for 14 days.[3]

In a recent study, phenylbutazone, flunixin meglumine and ketoprofen were administered intravenously and compared to controls.[90] Phenylbutazone caused the greatest amount of renal crest necrosis (3 of 3 horses) at 4.4 mg/kg IV, three times daily (TID), when compared to flunixin meglumine (1 of 5 horses) at 1.1 mg/kg IV, TID, and ketoprofen 2.2 mg/kg IV, TID, (0 of 5 horses).

Flunixin meglumine

Although gastrointestinal toxic effects were seen in one of the first reports of flunixin meglumine toxicity,[87] no adverse signs of renal toxicity were reported. In other toxicity studies, no renal or gastrointestinal toxicities were reported.[22] However, renal papillary necrosis was observed in several horses receiving varying doses of flunixin meglumine.[4] In this series of cases, many of the horses were receiving concurrent, potentially nephrotoxic, antibiotics as well as experiencing dehydration.

The effects of chronic flunixin meglumine therapy was examined in foals receiving recommended dosages for 30 days.[89] Gastrointestinal toxicity occurred without evidence of renal lesions. Another study examined the effects of flunixin at up to 6 times the recommended dose for 5 days.[102] No renal lesions were seen in this study. One foal in the 2.2 mg/kg group did experience acute renal failure. It was suggested that the renal failure in that foal was due to dehydration and diarrhea, although administration of the flunixin meglumine may have exacerbated the renal disease. In an attempt to measure effects of flunixin meglumine on renal blood flow, nonimaging nuclear medicine techniques were used in 6 healthy horses receiving 1 mg/kg twice daily for 3 treatments.[103] The results proved that flunixin meglumine at this dose does not significantly decrease renal blood flow. In a study comparing adverse effects of phenylbutazone,

flunixin and ketoprofen, one of 5 horses that received 1.1 mg/kg of flunixin meglumine every 8 hours developed renal crest necrosis.[90] In that horse no clinical signs of renal toxicity were apparent.

Ketoprofen

Since its approval in horses in 1990, ketoprofen has been used in only one toxicity study comparing the effects of phenylbutazone, flunixin meglumine, and ketoprofen.[90] No renal effects where noted in the horses treated with 2.2 mg/kg of ketoprofen every 8 hours.

Naproxen

In a study of continuous administration of naproxen to horses during training, no adverse effects of naproxen were seen.[104] However, this was not a toxicity study and to date, no toxicity studies have been performed in horses.

Biochemical Induced Changes in NSAID Toxicity

NSAID toxicity can cause changes in hemogram, serum enzymes, blood coagulation profile and urinalysis. The extent of the change depends on the nonsteroidal drug administered and the dose and duration of administration. Many studies in the horse have measured changes induced by NSAID toxicity.[3, 14, 19, 90] The values reported in some of these differ however, the alterations during toxicity are similar for most NSAIDs used in the equine.

As previously mentioned, some of the clinical signs associated with NSAID toxicity are depression, anorexia, diarrhea and shock. [3, 4, 5] An increase in packed cell volume (PCV), neutropenia, and a toxic left shift have also been noted in previous studies.[19, 102] These changes may be related to a suppression of granulopoiesis as has been seen in dogs [103] or from loss (destruction, consumption or sequestration) of circulating neutrophils.[104] Due to the gastrointestinal ulceration that is commonly induced during NSAID toxicity, it is believed that increased bacterial endotoxin absorption may occur and contribute to a toxic left shift.[18]

One of the earliest serum biochemical changes that occurs in NSAID toxicosis is a decrease in serum total protein and albumin concentrations.[14] These have been observed in both the presence and absence of gastrointestinal lesions.[14] Using radiolabeled

chromium chloride ($^{51}CrCl_3$), it was shown that protein loss from phenylbutazone toxicity occurs through gastrointestinal ulceration. Concurrent decreases in albumin also occur with NSAID induced toxicosis, presumably through the same mechanism of gastrointestinal loss or potentially through reduced synthesis within the liver.[57] Another potential cause of hypoproteinemia, which is more common in humans than in horses, is loss of protein in peripheral edema fluid that occurs in NSAID toxicity.[57]

During NSAID toxicity, decreases in protein may also cause a decrease in calcium due to reduced concentrations of protein-bound calcium, particularly albumin.[89, 105] Other biochemical values that may change in NSAID toxicity are increases in BUN, serum creatinine, and phosphorus concentrations due to medullary interstitial edema and tubular epithelial cell necrosis.[20] When phenylbutazone was administered to horses at doses of up to 30 mg/kg intravenously twice daily for two weeks no morphologic evidence of hepatotoxicity was noted and liver-specific enzymes (SDH, GGT) did not increase.[20] In man, however, elevation of these enzymes, as well as elevations of serum aspartate transaminase, are routinely observed.[106]

Changes in the urinalysis during NSAID toxicity are those that are directly related to renal papillary necrosis. These changes include isosthenuria with concurrent dehydration, proteinuria, an increase in granular cell casts and presence of blood in the urine.[5]

Changes in coagulation during NSAID therapy as well as NSAID induced toxicity have been observed in dogs,[106] horses [107, 108] and man.[109] Due to the inhibition of platelet cyclooxygenase and subsequent decreases in platelet activity, NSAIDs have the potential to increase bleeding times.[110] Platelet aggregation is thought to be initiated by adenosine diphosphate through specific receptors on the platelet surface. This causes calcium flux into the platelet and then subsequent contraction.[110] The aggregation response is then enhanced by secretion of products of the arachidonic acid metabolism from the platelets.[110] All NSAIDs have the ability to prolong or inhibit platelet aggregation; however, clinical effects are more common with acetylsalicylic acid rather than other NSAIDs. Acetylsalicylic acid binds irreversibly with platelet cyclo-oxygenase.[110]

Phenylbutazone

In an early study phenylbutazone was administered to horses in single doses from 1 to 16 grams with no significant alterations in the hemogram or chemical values.[81] Urinalysis and coagulation profiles were not performed in this study. In later two reviews of phenylbutazone in the horse following this study phenylbutazone was regarded as a safe NSAID. Furthermore, it was stated that there was a lack of evidence of biochemical alterations specifically in relation to hematological parameters,

serum proteins, creatinine, blood urea and aminoaspartate transferase changes.[3, 13] During the next ten years, 1977-1987, various studies subsequently revealed many biochemical changes associated with the administration of phenylbutazone.[3, 14, 18, 19, 57, 58, 114] One study found a decreased fecal excretion of chloride and an increased excretion of potassium in ponies when 1 gram of phenylbutazone was administered once daily for four days.[3] Also, this same dose caused no changes in packed cell volume, plasma sodium, potassium, carbon dioxide tension and chloride.[3] In a study in which 10-12 mg/kg of phenylbutazone was administered to ponies, drastic decreases in total plasma protein and albumin concentrations were concurrent with the finding of severe gastrointestinal ulcerations.[14] In another study, administration of large doses of phenylbutazone (up to 30 mg/kg orally or IV for two weeks), caused profound progressive neutropenia and a toxic left shift in all ponies.[19] Other changes seen in that study were significant progressive decreases in total serum protein accompanied with a decrease in serum calcium, increases in PCV, BUN, serum creatinine and serum phosphorus concentrations secondary to dehydration. In this same study, urinalysis examinations were unremarkable.[19] When attempting to define the "ideal" phenylbutazone dosage, phenylbutazone was given in doses of 4.4 mg/kg twice daily for four days, followed by 2.2 mg/kg twice daily for four days, then 2.2 mg/kg once daily for seven days.[85] Even using these decreasing doses, significant

decreases were still observed in serum total protein and albumin. However, no consistent changes were observed in cellular components of blood or fluid or in electrolyte balance during this system of administration.

In man, inhibition of prostaglandin interferes with antidiuretic hormone causing salt and water retention.[115, 116] Although phenylbutazone has been shown to interfere with the renin/angiotensin system in horses,[117-119] none of the toxicity studies performed in horses have demonstrated the fluid retention seen in people.[93]

In summary, phenylbutazone has been the most common nonsteroidal used in NSAID toxicity studies, and the biochemical changes exhibited vary depending on the dosage of drug used. The most sensitive biochemical indicator is total plasma or serum protein.[52] Other common findings in phenylbutazone toxicity in horses are a degenerative left shift, elevation in PCV (mainly due to diarrhea and dehydration), decreases in calcium and globulins and increases in BUN and creatinine due to renal papillary necrosis.[2, 5, 18] Occasionally a horse exhibits clinical signs of renal papillary necrosis, but very few changes in the urinalysis are observed.[5]

Flunixin meglumine

One of the first reports of flunixin meglumine toxicity was in a pony that had 3 mg/kg administered twice daily for 5 days.[87] Laboratory results revealed a progressive decrease in total protein up to Day 9 and then a slow rise that never reached baseline by Day 11 (day of euthanasia.) Other changes noted were a neutropenia and a decrease in albumin and calcium. This is in contrast to a toxicity study done by the manufacturer in which 3.3 mg/kg of flunixin meglumine was administered, once daily, intravenously for 10 days. In this study no clinical changes were observed nor were any changes noted in the hemogram, serum chemistries and urinalyses.[22] In a study examining the toxic side effects of flunixin meglumine in foals a dose of 1.1 mg/kg orally or intramuscularly, caused gastrointestinal lesions, however, no significant changes related to the drug were observed in the hemogram, serum biochemistry and fecal occult blood samples.[89] Another examined neonatal foals administered flunixin meglumine at dosages of 0.55 mg/kg, 1.1 mg/kg, 2.2 mg/kg, and 6.6 mg/kg intravenously for five days.[102] As in the study by Traub-Dargatz,[86] the gastrointestinal lesions were observed in the flunixin treated foals. Again, no statistically significant blood cellular or serum biochemical alterations were observed.[89] In the most recent study comparing the toxicity of flunixin meglumine, phenylbutazone and ketoprofen to saline in horses, flunixin

meglumine given in 1.1 mg/kg intravenously every 8 hours for 12 days did not cause any significant decreases in total protein or albumin concentration.[90] In this study there were also no change in urinalysis, urinary fractional clearance of electrolytes, or urinary gamma glutamyltransferase/ creatinine.

Ketoprofen

In a 15 day study, ketoprofen was administered at 1 mg/kg, 3 mg/kg, or 5 mg/kg (1, 3 and 5 times the recommended dose, respectively) and compared to a control.[120] Glandular ulceration developed but these were not different from lesions seen in controls. No changes were reported in blood chemistry, urinalysis, and hematology. Fecal occult blood was negative. In the same study 15 mg/lb or 25 mg/lb was administered intravenously once daily for 5 days. One horse administered 15 mg/lb had severe laminitis but apparently had no changes in laboratory data. The horse receiving 25 mg/lb had increased lactate dehydrogenase, SGOT, and GGT. In the study in which ketoprofen was administered to four horses at 2.2 mg/kg every 8 hours and compared to flunixin meglumine, phenylbutazone and a control, no gastrointestinal lesions were observed nor were any changes noted in total protein, albumin, WBC counts, urinalysis, urinary fractional clearance of electrolytes, or urinary gamma glutamyltransferase/creatinine.[90]

Naproxen

Naproxen has most commonly been administered to horses that have undergone mild to severe myositis (tying up). In clinical efficacy studies no toxic side effects have been observed when administered at the recommended dose (4 grams orally, twice daily).[104] When bleeding times were studied in horses administered naproxen at 5 mg/kg intravenously no changes were observed.[121] No change in clotting time was observed when measured using naproxen in vitro.[122]

Less commonly observed NSAID-induced side effects

Jugular vein thrombosis has been associated with the intravenous administration of phenylbutazone.[12, 52] Usually the reactions are associated with subcutaneous or perivascular injection which results in inflammation, abscessation, thrombophlebitis and eventually sloughing of the vein.[121] None of the other NSAIDs in this review (flunixin meglumine, ketoprofen, and dipyrone) have been associated with this finding.[121]

Necrotizing phlebitis of the portal veins has also been reported with phenylbutazone toxicity in horses.[81] It has been hypothesized that portal phlebitis is more common after orally administered phenylbutazone due to a potentially higher concentration being present in the portal venous concentration[19] as compared to parental administration. Experimental data are, however, lacking.

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Table 1. CHEMICAL CLASSIFICATION OF NSAIDS USED IN EQUINE PRACTICE (From Kallings, P: Vet Clin NA Eq Prac, 9, 524, 1993)

Enolic Acids	**Carboxylic Acids**
Pyrazolones	*Salicylates*
Phenylbutazone*	Acetylsalicylic Acid
Oxyphenbutazone	Methylsalicylate
Isopyrine	*Propionic acids*
Dipyrone	**Naproxen**
	Ibuprofen
	Ketoprofen
	Anthranilic acids
	Meclofenamic acid
	Aminonicotinic acids
	Flunixin meglumine
	Indolines
	Indomethacin

*The drugs in bold print are reviewed in this literature review.

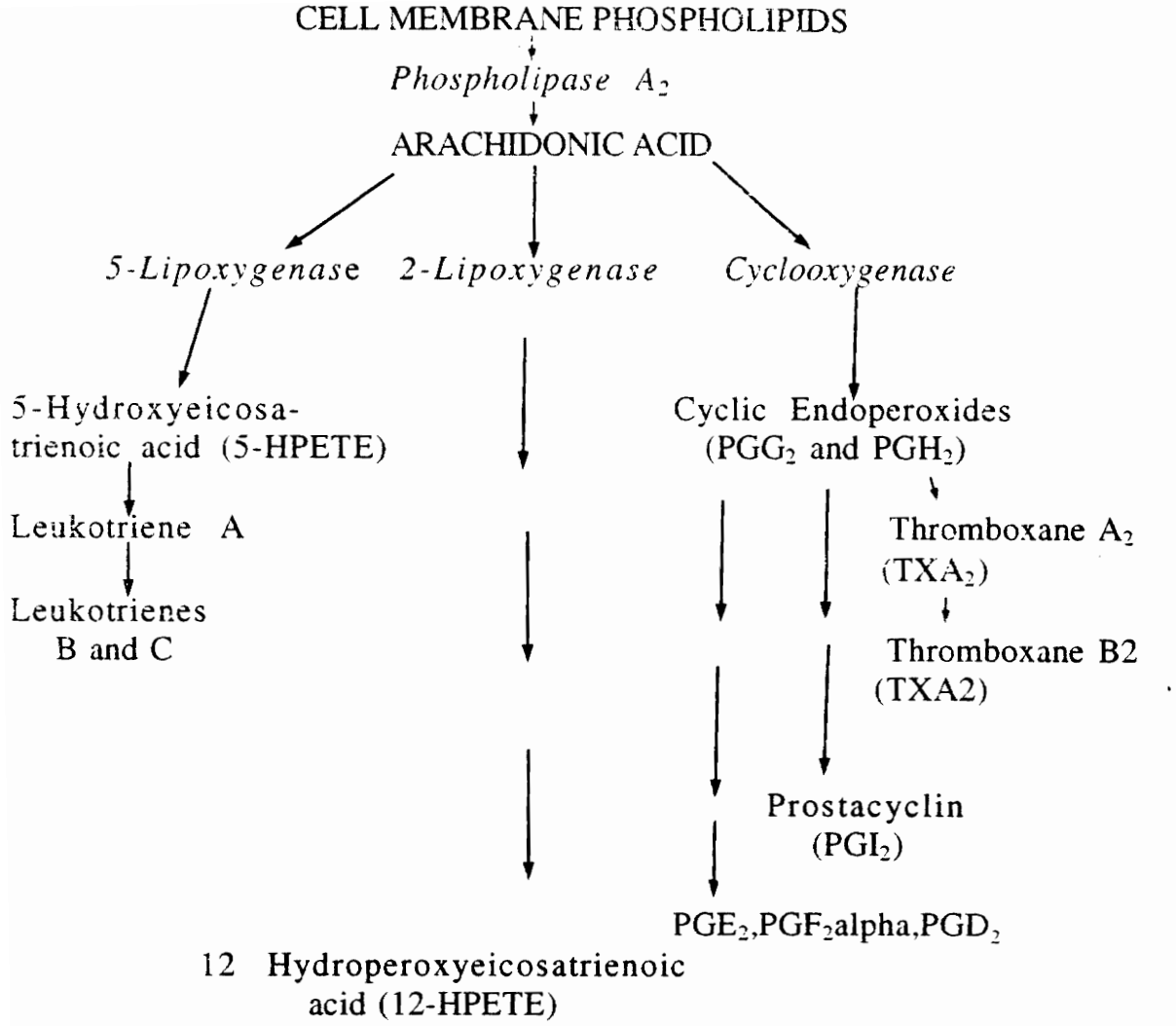

Figure 1. The Formation of Eicosanoids Via the Arachidonic Acid Cascade (From Miller, TA: Am J Physiol 245, G601-G623, 1983)

A TOXICITY STUDY OF ELTENAC (4-[(2,6-dichlorophenyl) amino]-3-thiophene acetic acid), A NOVEL NONSTEROIDAL ANTI-INFLAMMATORY DRUG, IN HORSES

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Introduction

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) in horses is extremely common due to their beneficial anti-inflammatory, analgesic and antipyretic effects. Their primary mode of action is inhibition of prostaglandin biosynthesis. Unfortunately, the use of some NSAIDs in horses may cause gastrointestinal erosion, ulceration, diarrhea, anorexia, depression[1] or renal papillary necrosis.[2]

Only a small number of NSAIDs are approved for use in horses. Species variation in toxicity, bioavailibility and biotransformation narrows the safety of these drugs in horses. One of the most commonly used NSAIDs, phenylbutazone, is toxic when administered at levels close to the therapeutic range.[1, 3, 4] Given the gastrointestinal and renal toxicity caused by NSAIDs in horses, studies examining the clinical and toxicological effects are necessary before the drug is put into clinical use.[5]

Eltenac, 4-(2,6-dichloroanilino)-3-thiophene acetic acid, is a cyclo-oxygenase inhibitor. In a previous clinical efficacy study eltenac was administered intravenously at 1 mg/kg every 24 hours for seven days to horses with either musculoskeletal pain or post-operative wound swelling.[6] In horses exhibiting musculoskeletal pain, significant analgesia was seen for a 24-hour period following a single intravenous injection. In horses with post-operative wound swelling, edema was significantly reduced. These results demonstrated the drug to be an effective analgesic and anti-

inflammatory compound. No adverse reactions were noted over a period of seven days.

The purpose of the current study was to determine the potential toxic effects of eltenac when administered intravenously to horses at a dose rate of 0.5 mg/kg (anticipated clinical dose), 1.5mg/kg and 2.5mg/kg once daily for 15 days.

Materials and Methods

Horses

Twenty-four mixed breed horses (12 neutered males, 12 non-gravid females) were obtained from a commercial livestock dealer. The horses were determined to be healthy by physical examination before being accepted for the study. Horses were 2-8 years of age, as judged by dentition, and weighed between 325-500 kg. Mares were determined to be nongravid by rectal exam. Routine physical examination, urinalysis (urine collected via catheterization), complete blood count and serum chemistry were performed. All horses were dewormed with 0.2 mg/kg of oral ivermectin[a] and vaccinated against tetanus, and equine eastern/western encephalomyelitis,[b] equine rhinopneumonitis and equine influenza.[c]

Assignments, housing and dosing

Horses were assigned to pens by body weight rank order. All horses were weighed and then ranked from heaviest to lightest within sex. Horses were then assigned to 4 pens in the order 1,2,3,4,4,3,2,1,1,2,3,4 (3 treatment groups and one control) in order

to balance the distribution of body weights in each group. This resulted in 3 mares and 3 geldings in each pen and a similar distribution of body weights among groups. Horses were acclimated to study conditions for 14 days (Day -14 to Day -1) prior to the commencement of dosing with eltenac.

Horses were housed in one of four adjacent 50 x 100 ft. open pens. Each pen was equipped with an enclosure for shelter during inclement weather. Ambient outdoor temperatures and weather conditions were recorded on a daily basis. Horses were fed a commercial grain mixture[a] at a rate of 2 pounds per horse, divided into two feedings (AM, PM) and were provided free choice hay. The amount of grain consumed by individual horses was not quantified. However, appetite and food consumption were subjectively assessed each day. Hay consumption and water intake were measured daily for each pen.

Dosing

The dose level of eltenac was randomly assigned by pen. Horses in pen #2 received sterile saline, pen #1 received 0.5mg/kg of eltenac, pen #4 received 1.5 mg/kg of eltenac, and pen #3 received 2.5 mg/kg of eltenac. A sterile solution of eltenac[•] (50mg/ml), in multidose vials was provided by the manufacturer. Eltenac or sterile saline was administered intravenously for a period of 15 days. Injections were given in jugular veins, alternating daily between left and right veins. The volume of solution administered equaled: body

weight (kg) x dose level/50mg/ml. Horses in the control group received a volume of saline equivalent to horses dosed with eltenac.

Evaluations during the study

Beginning on Day -15 (fifteen days prior to dosing), observations were made twice daily. Observations included condition of the eyes and integument, heart rate, respiratory rate, fecal consistency, and presence or absence of lameness. Hydration was estimated through observation of skin turgor and mucous membranes. Discharges, congestion, or gross lesions of the eyes were noted. Pulse rate and quality were determined by palpation of the facial artery where it traverses the ventral mandible. The rate of respiration and respiratory effort were assessed visually. The attitude, behavior and equilibrium of each horse was observed and abnormalities noted.

Body weights were determined at the time of arrival and on Days -23, 1, 5, 10 and 15. A complete physical examination of each horse was done on Day -21 and on Days -1, 5, 10, and 15 (physical examinations were much more complete examinations than daily observations). Routine ophthalmic exams were done on Day -1 and Day 15.

An endoscopic examination of the oropharynx, nasopharynx, esophagus, and stomach of each horse was done once during acclimation (Day -8), and on Days 7 and 12 of dosing. This examination was performed following a 12-hour fast. The gastric

squamous area and glandular fundus of each horse were visually examined and photographed for later computer-assisted analysis.

Clinical pathology

Jugular venous blood samples, urine (collected by catheterization) and feces were collected from all horses on study Days -1, 3, 6, 10, and 15. Whole blood samples were collected by jugular venipuncture into vacuum-evacuated blood tubes containing .06ml of 75% EDTA (K3) or glass tubes without anticoagulant[f] for determination of complete blood and platelet counts, and serum chemistry analysis, respectively. Whole blood was also collected into blood tubes containing buffered sodium citrate[g] for coagulation analysis. Coagulation analysis evaluated prothrombin time, activated prothrombin time and fibrinogen using test reagents.[h] Complete blood counts were analyzed by automated method.[i] Differential white blood cell count was determined by standard microscopic methods. Serum and electrolyte parameters were measured using automated methods.[j] Analytes asssayed were glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, phosphorus, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, creatinine kinase, blood urea nitrogen/creatinine ratio, direct bilirubin, indirect bilirubin, magnesium, and osmolality.

Urine for analysis was collected by catheterization. Values assessed and methods used included: specific gravity and refractive index via a refractometer,[k] protein, ketones, bilirubin, glucose, blood, pH[l] and analysis of sediments (WBC's, bacteria, crystals, amorphous sediment, casts, and renal and squamous epithelial cells) via microscopic examination.

Feces were collected directly from the rectum of each horse for assessment of parasite burden and fecal occult blood. Fecal occult blood was measured using a stool guaiac method.[m]

Necropsy

On Day 15 all horses were killed with concentrated barbiturate solution[n] and a complete postmortem examination was performed.

Since many NSAIDs in common use cause gastric irritation, stomachs were prepared for special examination after fixation. The stomach was removed, tied with string at the base of the pyloric antrum and filled with neutral buffered 10% formalin solution through a funnel in the esophagus. The esophagus was then ligated and the entire formalin-inflated stomach was immersed in a container of formalin.

A panel of tissues were collected for microscopic examination. These were placed in formalin fixative for a minimum of 48 hours and then trimmed for histologic processing. All processed tissues were stained with hemotoxylin-eosin stain and examined by a pathologist who had no knowledge of assignments to treatment groups. Tissues examined histologically were: adrenal glands, aorta,

bone marrow, brain, cecum, colon, diaphragm, duodenum, esophagus, eyes, heart, ileum, jejunum, liver, lung, lymph nodes, pancreas, parathyroid, pituitary, salivary gland, skeletal muscle, skin, spleen, spinal cord, stomach, thymus, thyroid, tongue, trachea, urethra, and urinary bladder. In addition mammary gland, ovary, uterus, and vagina of females and the prostate (when found) of males were examined histologically.

All daily observations and physical examinations, gastroscopy, and post mortem examinations were performed by individuals that were unaware of assignment and dosage levels administered. To further eliminate any subjective bias, daily clinical assessment of horses were made by individuals different from those that performed the physical examinations.

Statistical analysis

The effect of dose group on the change in continuous response variables (serum chemistry, hematology, temperature, pulse and respiration, and some urinalysis measurements) over time was tested using repeated measures analysis of variance (ANOVA). For variables where the change in measurements were significantly affected by dose group ($P < 0.05$), separate polynomial regression models for each time after beginning treatment were used to determine if there was a linear or quadratic relationship between dose and the change in the measurement from baseline. For these regression models, the response variable was the difference between the measurement on a particular day of treatment and the

pretreatment measurement for each horse. Normal probability plots of residual values and plots of residuals against predicted values were examined to evaluate model assumptions. The general linear models procedure of SAS®[o] was used for the analyses involving continuous response variables.

For daily clinical observations, physical examination findings, and gastroscopy, horses were considered to be affected by a particular type of lesion or condition if it was diagnosed at least once after treatment and was not present before the treatment period. The effect of increasing dose on the occurrence of these abnormal findings was tested using exact trend tests with drug doses entered as scores from 1 through 4.[p] Conditions with increased incidence in higher dose groups and a statistically significant linear relationship with drug dose group ($P < 0.05$) were considered to be associated with drug treatment.

Results

Daily observations, food and water consumption; body weights

None of the horses receiving intravenous sterile saline, or eltenac at 0.5mg/kg, 1.5mg/kg or 2.5mg/kg exhibited any change in attitude throughout the study. No signs of anorexia or colic were observed and appetites remained constant.

Isolated clinical changes were noted sporadically in 6 horses in different groups. Changes observed were; epiphora (1 horse receiving saline, Day 7), conjunctivitis (one horse, receiving 1.5

mg/kg of eltenac, Day 11 and 12), diarrhea (one horse, receiving 0.5 mg/kg of eltenac, Day 4), lameness associated with a foot abscess (one horse, receiving 0.5 mg/kg of eltenac, Day 7 and Day 11-15), and dermatitis (two horses receiving 2.5 mg/kg of eltenac, Day 5-7 and Day 1-14, respectively). None of these changes were found to be clinically significant and none were judged to be associated with administration of the drug.

Water consumption per pen varied with daily temperature but pens of horses receiving doses of eltenac were similar to saline treated horses. Hay consumption was also similar between pens. Neither hay nor water consumption were analyzed statistically. Mean body weights for the horses in each pen were between 408 lbs and 427 lbs. Body weight were not affected by saline or administration of the drug.

Physical examination

Changes found during complete physical examinations from pretreatment (-1) to post treatment (Day 5, 10, or 15) are recorded in Table 1.

Oral ulcerations were present on the tongue or gums of 5 horses. These were small, singular lesions. Soft fecal consistency was noted in one of the horses treated with 1.5 mg/kg of eltenac and one of the horses treated with 2.5 mg/kg of eltenac on one occasion respectively. However, diarrhea was not observed in either one of these horses nor were any other physical examination abnormalities. Ventral abdominal edema was noted in one horse administered 2.5

mg/kg of eltenac. Tachypnea and tachycardia was noted in several horses receiving saline or eltenac. These signs were usually associated with restraint and required several minutes for heart rate and respiration to decrease. These changes were not associated with abnormal auscultation of either the heart or lungs. Ophthalmic examinations on Day -7 and Day 15 were normal for all horses.

Jugular vein thickening associated with repetitive injection was observed in a few horses in each group. This generally was due to an increased thickness of tissue over the jugular veins. However no heat, pain or swelling was associated with the areas. None of these changes were significant or related to dosing with eltenac. Two horses did exhibit swelling and heat in the injection site area, one of the horses was in the saline group and the other was receiving 2.5 mg/kg of eltenac.

Gastroscopy

Gastroendoscopic changes seen are described in Table 2. Minimal focal lesions of the squamous mucosa developed in 3 of horses administered 0.5 mg/kg and one horse administered 2.5 mg/kg of eltenac. No squamous mucosal lesions developed in horses administered saline or 1.5 mg/kg of eltenac. The changes seen in the squamous mucosa were not significant.

Glandular lesions in the fundus and pylorus developed only in horses treated with eltenac (1 horse administered 0.5 mg/kg, 3 horses administered 1.5 mg/kg and 3 horses administered 2.5 mg/kg) of eltenac. One of the horses receiving 1.5 mg/kg of eltenac

developed a lesion (patchy reddening) on Day 7 which had resolved by Day 12. There was a significant relationship between increasing dose of eltenac and the proportion of horses which developed glandular lesions as seen on gastroscopy ($p = 0.02$).

Hematology

The hematological and coagulation parameters that changed significantly are listed in Table 3. The significant ($p < 0.0001$) effect of dose on the change in WBC counts over time appeared to be due to an initial decline in neutrophil counts on days 3 and 6 for the horses administered 1.5 mg/kg and 2.5 mg/kg of eltenac (Fig. 1). Four of six horses receiving 2.5 mg/kg of eltenac had a neutropenia (group mean of 1782 cells/ul) on Day 6 of testing that rebounded at the following testing periods (Fig. 2). Horses receiving 1.5 mg/kg also had a decrease in neutrophil count but the mean did not fall below 2000 cells/ul.

All group mean values for RBC, HGB and HCT as well as values for individual horses were within normal limits. Although repeated measures ANOVA found a statistically significant effect of grouping on changes in RBC, HGB and HCT over time ($P < .05$), the changes in these values did not have a significant relationship with increasing drug dose. This was also the case for prothrombin, activated partial thrombin time and fibrinogen.

Blood chemistry values

Serum biochemical values that changed significantly are listed in Table 4. The changes in total protein values were associated with

drug dosage level ($p < 0.0001$) (Fig. 3). The protein values for the saline treated group remained similar to baseline pretreatment values. Total protein for the horses treated with 0.5 mg/kg and 1.5 mg/kg eltenac increased slightly on Day 3 but then decreased. Horses treated with 2.5 mg/kg of eltenac decreased within the first 3 days and continued to decrease, having the lowest mean total protein of all groups by the end of the study. Mean albumin levels for saline treated horses and horses receiving 0.5 mg/kg of eltenac remained similar throughout the dosing periods. However, horses receiving 1.5 mg/kg and 2.5 mg/kg of eltenac had a decrease in albumin significantly ($p < 0.05$) relative to dose level at each time of testing throughout the study. Globulin values for saline treated horses remained within normal ranges for horses of this age. Mean globulin values significantly decreased ($p < .05$) for horses receiving 0.5 mg/kg, 1.5 mg/kg and 2.5 mg/kg of eltenac. The 2.5 mg/kg dose horses had the lowest mean globulin value on Day 15.

Repeated measures ANOVA results showed significant treatment group effects on the change in blood concentrations during the study of mean aspartate aminotransferase, BUN, creatinine, GGT, calcium, magnesium, phosphorus, chloride, and sodium. However, these changes were not found to be related to the dose of eltenac administered.

There were no drug-related changes in urinalysis values. Presence of fecal occult blood did not differ significantly between the

horses treated with saline and the horses treated with either 0.5 mg/kg, 1.5 mg/kg or 2.5 mg/kg of eltenac.

Pathology findings

Post mortem findings are summarized in Tables 5, 6, and 7. None of the gross post mortem changes were clinically important or associated with drug dose. Lesions within the oral cavity were found in some eltenac treated horses. Lesions were either located on the tongue or lip and each horse had only one lesion. All were confirmed histologically as either minimal focal erosions or ulcerations.

Stomach erosions/ulcerations were seen in the nonglandular or glandular portions of the stomach. Nonglandular (squamous mucosal) erosions/ulcerations were seen in none of the horses administered saline or 0.5 mg/kg of eltenac, in three of horses administered 1.5 mg/kg and in one horse administered 2.5 mg/kg of eltenac. Glandular lesions were seen in one horse administered saline and one horse administered 0.5 mg/kg, two horses administered 1.5 mg/kg and three horses administered 2.5 mg/kg of eltenac. There was a dose-related trend for glandular lesions as dose increased. However, this change in lesion incidence was not found to be statistically significant ($p = 0.12$). Histologically, all lesions except for horse #16 were classified as erosions and not as ulcerations.

Gross lesions of the small intestine, large intestine, cecum and small colon are listed in Table 6. These changes were considered incidental and not related to administration of drug.

Respiratory lesions consistent with inflammation were seen grossly in horses in all groups (Table 7). These changes were confirmed with histology as minimal to moderate bronchiolitis and were not found to be clinically significant or dose-related. No significant renal changes were seen in any horses.

Jugular vein lesions were present in a few horses (Table 7). These lesions were considered to be related to repeated intravenous injections and not to dosing with eltenac. No abscessation, sloughing or thrombosis was observed.

Other spontaneous and incidental (not related to drug treatment) pathological findings included mucosal hyperemia of the urinary bladder, dermatitis, pericardial adhesions, splenic congestion and lymph node hyperplasia.

Discussion

Clinical changes due to nonsteroidal toxicity in horses have been well documented.[1, 4, 6-11] Initial clinical signs may include inappetence and depressed attitude but may progress to diarrhea, oral and gastrointestinal ulceration and a decrease in weight. [8] Animals displaying extreme toxicity may suffer elevated heart rate, cold extremities, decreased body temperature and ultimately death.[1, 3, 7] Horses of this study treated with eltenac, remained alert and did not experience a decrease in appetite or body weight. One of the horses treated with the lowest dose of eltenac had diarrhea on Day 4 of the study. However, no other parameters were changed in this horse, and the diarrhea resolved spontaneously over

a period of 24 hours. Two other horses, one treated with 1.5 mg/kg of eltenac and one treated with 2.5 mg/kg of eltenac, were noted to have soft feces on physical examination on Day 15. The feces were not considered diarrhea and were not believed to be related to a treatment effect.

No treatment-related oral lesions were observed. It has been suggested in human studies that oral preparations of NSAIDs are more ulcerogenic to oral mucous membranes than intravenous preparations.[12] However, studies in horses given toxic doses of nonsteroidals showed no differences in the occurrence of oral ulceration by either intravenous or oral route of administration.[12] The oral ulcerations in horses in this study were considered mild and incidental and appeared to be associated with scratches from hay rather than NSAID toxicity.

One of the two horses that developed loose stool also had ventral edema on the abdomen. The edema was associated with a low total serum protein of 3.4 g/dl. The reason for a low total protein in this horse as well other horses receiving 1.5 and 2.5 mg/kg of eltenac was not documented. Previous studies have documented protein losing enteropathy induced by phenylbutazone using $^{51}CrCl$ labeled albumin measurement in the feces.[3] Since this was not performed in this study, the cause of decreased protein is speculative.

Perivascular leakage following injection of other NSAIDs such as phenylbutazone has been associated with phlebitis and necrosis.[14]

In this study, jugular vein and peripheral tissue thickening (fibrosis) occurred in horses in both saline and eltenac treated horses despite alternating injection sites between left and right jugular veins. It was not associated with heat, pain, or swelling of the injection site. We assume that thickening observed at injection sites was the result of repeated venipuncture. Two horses had obvious heat, pain and swelling at the site of injection. One of these horses had an indwelling venous catheter at this site, which was needed to facilitate injection of drug due to the animal's behavior. The other horse developed phlebitis (confirmed histologically). The sporadic occurrence of injection site lesions, lack of dose-related trends in incidence, and our clinical experience suggests that eltenac is not toxic to tissue at the site of intravenous injection.

The development of gastric ulceration is a common sign of NSAID toxicity.[1, 3, 12, 15, 16] Using gastroendoscopy in this study, we found lesions in both the squamous and glandular portions of the stomachs in several horses. Only those of the glandular stomach appeared to be related to dosage level and these changes were minimal. Other studies of NSAID toxicity found that development of gastric lesions was usually more severe in glandular than nonglandular regions of the stomach.[1, 3, 12, 16] However, not all gastric lesions in horses are related to NSAID therapy. The occurrence of incidental squamous ulcerations in normal horses was found to be relatively common. Murray[17] found such lesions in 52% of horses examined. Another study found gastric ulcerations in 66%

of 195 race horses necropsied, presumably related to the stresses of training and competition.[18] It is possible that the squamous lesions observed in this study were spontaneous.

Gastric lesions seen at necropsy were mild in severity. Glandular mucosal lesions were all classified as erosions. The occurrence of glandular lesions increased as dose increased presumably due to a differential effect of the drug on glandular mucosa. NSAID-induced gastric lesions have been observed more commonly at necropsy in glandular mucosa rather than squamous mucosa.[10, 16, 19] Three of the horses that had confirmed glandular lesions via gastroscopy on day 12 had similar lesions described at necropsy (horses 15, 22, and 24). Two additional horses, that also had glandular lesions confirmed with gastroscopy, worsened in severity from gastroscopy at Day 12 to necropsy (horses 7 and 18). The consistency of lesions seen both by gastroscopy and on necropsy confirmed the value of gastroscopy in the diagnosis of gastric ulceration during the course of NSAID toxicity studies.

It was of interest to us that while the incidence of glandular mucosal lesions was statistically significant in the antemortem period (by gastroscopy), this trend was not seen with data gathered at necropsy. The difference between gastroscopic results and postmortem results is due to the healing of lesions in 1 horse (horse 17) during the treatment period and the development of a glandular lesion in a saline treated horse. This the healing of the lesion suggests there may be accommodation to the presence of the drug.

Adaptation of gastric mucosa to NSAIDs has been reported in man [13] however the mechanism is unknown. The development of a glandular lesion in the saline treated horse suggests that some lesions were spontaneous and not associated with the administration of eltenac.

The occurrence of neutropenia seen in this study as a result of dosing with an NSAID is consistent with data from other studies, [11, 14] where the development of a reversible neutropenia was observed. In this study, the cause of neutropenia was not determined. There were no significant inflammatory lesions observed in any of the tissues examined or evidence of bone marrow suppression. An intermittent neutropenia has been reported as a manifestation of NSAID toxicity in dogs,[22] but this has not been reported in horses.

Total serum protein decreased in a dose-dependent fashion. This was a mild panhypoproteinemia reflected by both an albumin and globulin decrease. These lowered values could have been from losses into the gastrointestinal tract, into the urine, or decreased synthesis. However, there was no significant increases of protein in the urine at the time of peripheral hypoproteinemia. A protein-losing gastroenteropathy has been documented in toxicity studies of phenylbutazone in the horse.[1, 12, 23] Fecal losses of plasma albumin, using a 51Cr-labeled plasma albumin technique, were shown in ponies after administering large doses of phenylbutazone (8 -12 mg/kg for 8 days).[3] The horses in our study did not have

lesions on necropsy consistent with protein-losing enteritis. Other studies of NSAID toxicity have reported that gastrointestinal loss of protein without visible ulceration [3, 15] and this is a possible explanation of our findings.

Eltenac inhibits the production of cyclooxygenase, thereby inhibiting prostaglandin production.[16] Prostaglandins may protect the gastric mucosa against injury induced by NSAIDs. This protective mechanism, cytoprotection, has been studied extensively in many species.[6, 17-20] Prostaglandins may increase gastric blood flow, mucus, bicarbonate, and fluid secretion, and increase ionic transport, cyclic AMP and surface-active phospholipids.[21] In the gastric mucosa, the most common prostaglandins found are prostacylin (PGI) and PGE_2.[22] Within the stomach, these prostaglandins play an important role in maintaining mucosal vascular integrity,[22] as well as maintaining water and electrolyte absorption, accelerating ulcer healing and exerting cytoprotection.[31-33] Experimentally-induced phenylbutazone toxicosis in ponies has been prevented with synthetic prostaglandin E_2.[6] There have been other studies that question the importance of prostaglandin levels in NSAID toxicosis. In those studies, assays measured prostacylin and prostaglandin E_2 concentration in NSAID-induced gastric,[16] and intestinal ulceration with no significant differences observed before or after induced ulceration. [34] In both studies, it was concluded that phenylbutazone-induced gastric and intestinal erosions were due to direct toxic effects of the drug on the endothelium of the vasculature

and the mucosa. It was felt that the toxic effects of NSAIDs were mediated through prostaglandin independant pathways.

There are a limited number of NSAIDs available for use in the horse. Toxic side effects have been observed at 2-3 times the effective (therapeutic dose).[35] In one study, three commonly used NSAIDs, phenylbutazone, flunixin meglumine and ketoprofen, were compared to a saline treated group for adverse side effects.[36] Phenylbutazone was administered at 1.5 x's the recommended initial dose, flunixin meglumine was administered at 3 x's the recommended dose, and ketoprofen was administered at 3 x's the recommended dose, for 12 days, respectively. Gastric glandular erosions associated with drug treatment were found in all NSAID treated horses. A significant relationship was seen between increasing drug dose and lesion severity in the phenylbutazone and flunixin meglumine treated horses. Post-mortem examination of the horses in our study showed no statistically significant differences in stomach glandular mucosa lesions between horses treated with saline and horses treated with up to 2.5 mg/kg (5 times the anticipated clinical dose) of eltenac. However, a trend existed for more stomach glandular mucosal lesions to occur as the dose of eltenac increased ($p = .12$).

In the study comparing saline to phenylbutazone, flunixin meglumine, and ketoprofen, significant decreases in total protein were detected only for the horses given phenylbutazone.[23] In our study, higher doses of eltenac resulted in decreased total protein as

evidenced by 6 of 6 horses in the 2.5 mg/kg group exhibiting hypoproteinemia by Day 15.

In conclusion, eltenac did not induce gastrointestinal injury at a dose of 0.5 mg/kg intravenously once daily for 15 days and causes minimal gastrointestinal injury at 1.5 mg/kg and 2.5 mg/kg for the same dose period. The adverse effects that where seen with eltenac at the intravenous dose of 1.5 mg/kg and 2.5 mg/kg were a decrease in total protein, albumin and globulin and mild neutropenia. Eltenac administered at 0.5 mg/kg, 1.5 mg/kg and 2.5 mg/kg intravenously for 15 days does not cause any adverse effects in physical examination findings. Since previous reports support eltenac's anti-inflammatory properties it appears to be a useful and relatively non-toxic compound.

Further studies are needed to determine why eltenac is less toxic in the horse than other currently used NSAIDs. A study examining the effects of eltenac, and other commonly used NSAIDs, on the GI mucosal prostaglandin levels would potentially help the clinician understand if gastrointestinal toxicity is, in fact related to mucosal prostaglandin levels. Furthermore, it would be interesting to determine if eltenac has any selectivity on the two known forms of cyclooxygenase enzymes to determine if this too has a bearing ondecreased toxicity. If the exact mechanisms of toxicity could be defined, then NSAIDs could be better formulated to provide maximal efficacy and minimal toxicity.

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[a] Eqvalan, Merk and Co. Inc., Rahway, NJ
[b] Equiloid, Fort Dodge Laboratories, Inc., Fort Dodge, IA
[c] Fluvac EHV 4/1, Fort Dodge Laboratories, Fort Dodge, Inc., IA
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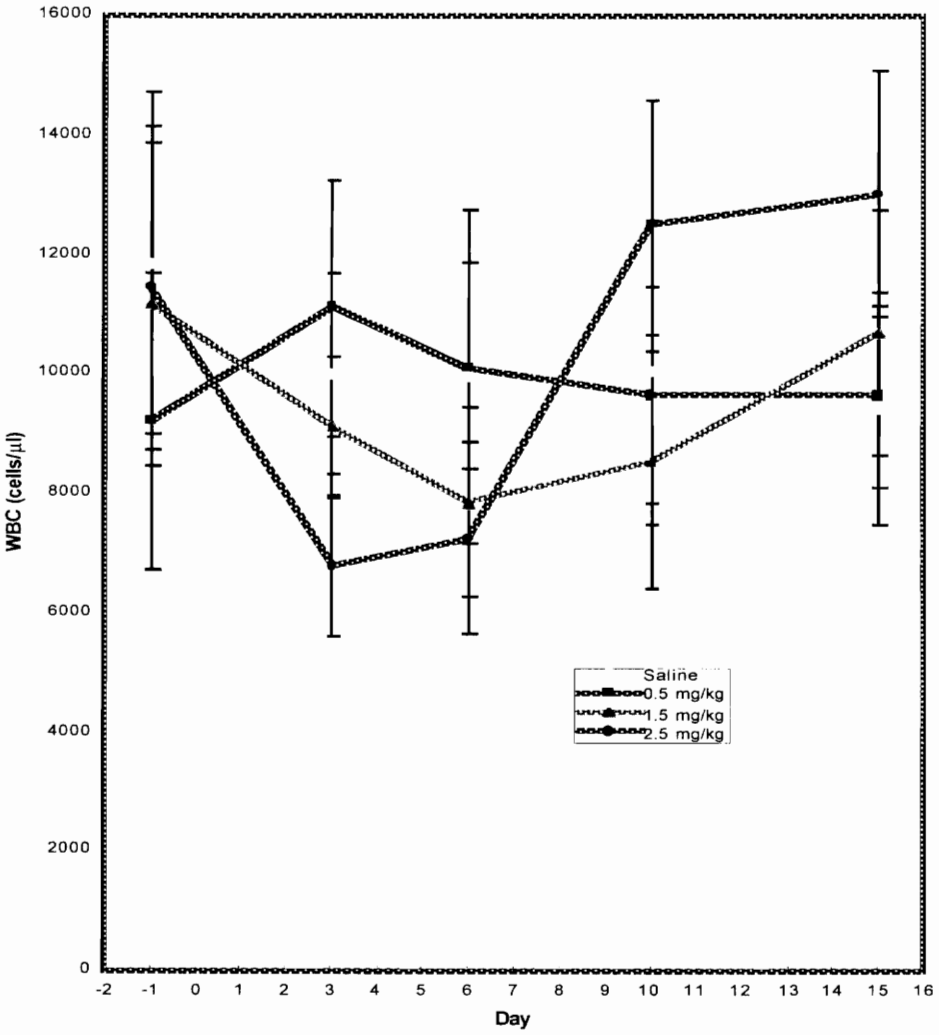

Figure 1 - Graph depicting mean white blood cell counts over time for groups of horses receiving daily intravenous injections of either sterile saline, or eltenac at 0.5 mg/kg, 1.5 mg/kg or 2.5 mg/kg beginning on Day 0 (error bars stand for 2 x's the standard deviation of the mean).

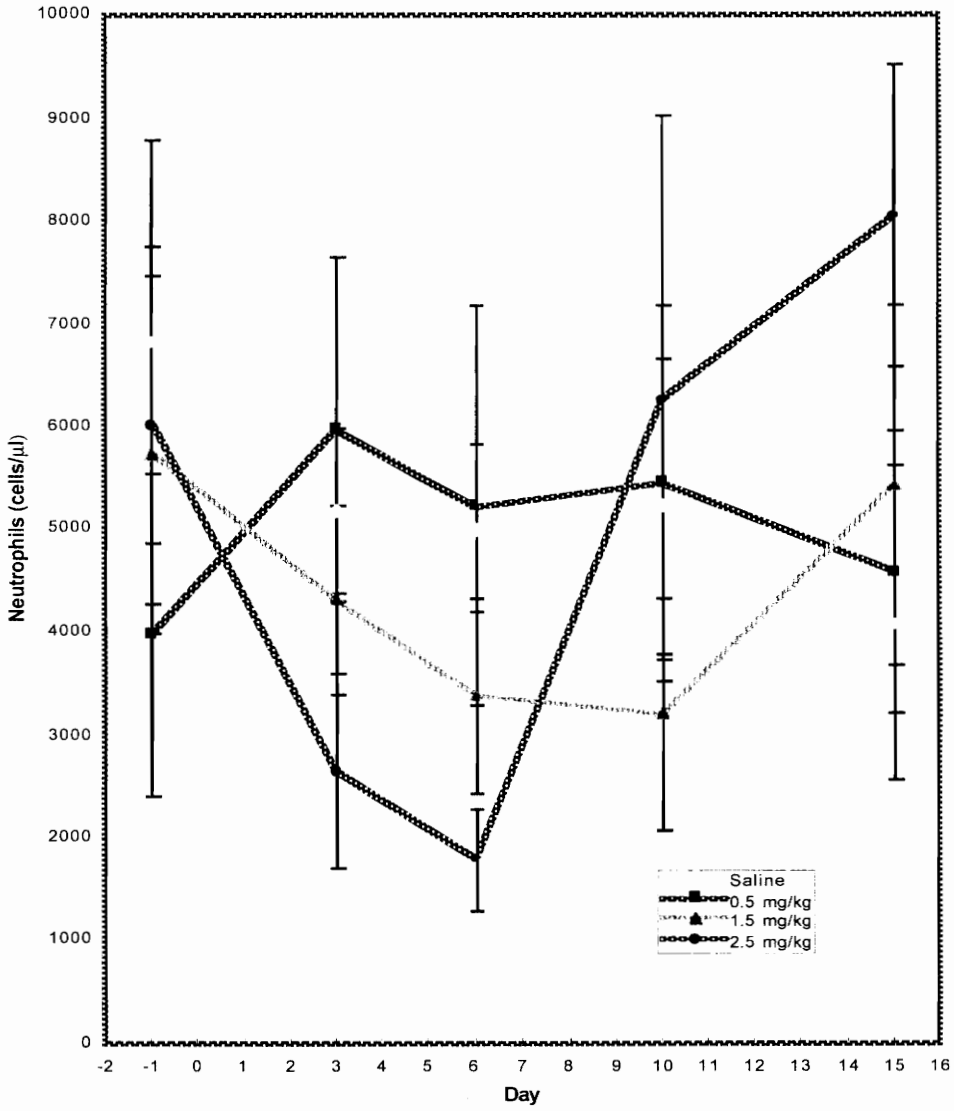

Figure 2 - Graph depicting mean neutrophil counts over time for groups of horses receiving daily intravenous injections of either sterile saline, or eltenac at 0.5 mg/kg, 1.5 mg/kg or 2.5 mg/kg beginning on Day 0 (error bars stand for 2 x's the standard deviation of the mean).

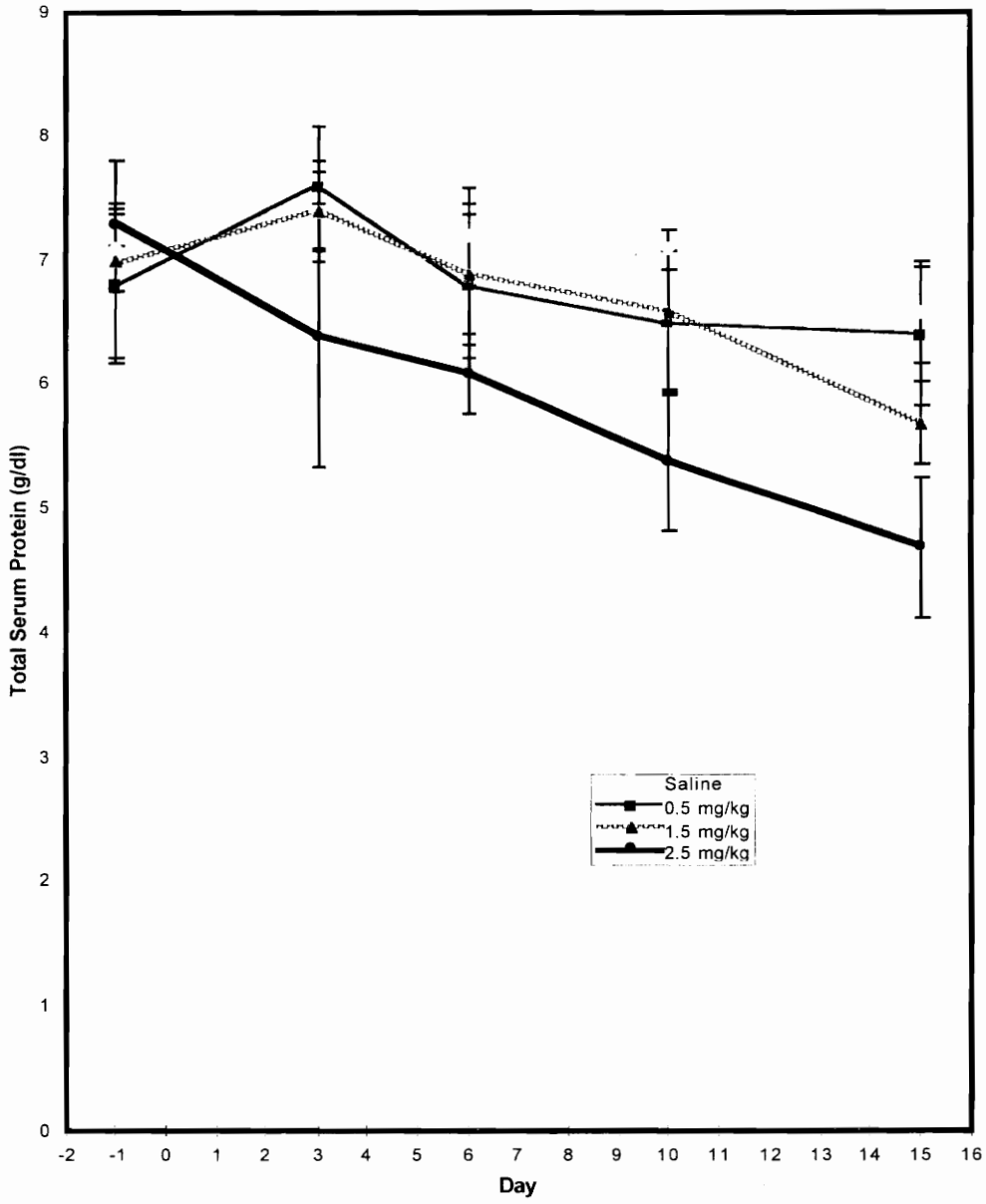

Figure 3 - Graph depicting mean neutrophil counts over time for groups of horses receiving daily intravenous injections of either sterile saline, or eltenac at 0.5 mg/kg, 1.5 mg/kg or 2.5 mg/kg beginning on Day 0 (error bars stand for 2 x's the standard deviation of the mean).

Table 1 - Results of changes in physical examination findings from predosing to days 5, 10, and 15 for horses administered intravenous sterile saline, or eltenac at .5mg/kg, 1.5mg/kg or 2.5mg/kg once daily for 15 days. None of the changes were found to be associated with drug dose (recorded as day the physical examination abnormality was seen).

Horse	Saline or eltenac (mg/kg)	Cough	Oral Ulcer	Loose stool	Ventral edema	Alopecia	Tachy-pnea	Nasal discharge	Tachy cardia	Jug vein thickening	Jug vein phlebitis	Sinus arrythmia	Murmur	Lip ulceration
1	saline	-	-	-	-	-	5	10,15	-	-	-	-	-	-
2	saline	-	-	-	-	-	5	5	10	15	-	-	-	-
3	saline	-	-	-	-	-	-	-	-	-	-	-	-	-
4	saline	-	-	-	-	10	10	15	-	-	10	10	5	-
5	saline	-	5,10	-	-	-	5	-	-	-	-	-	-	-
6	saline	-	-	-	-	-	-	-	-	15	-	-	15	-
7	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
8	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
9	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-
10	0.5	-	-	-	-	-	5	-	-	10,15	-	-		-
11	0.5	15	5	-	-	-	-	15	-	5	-	-	-	-
12	0.5	-	5,10	-	-	-	5	-	5	-	-	-	-	-
13	1.5	-	-	15	-	-	-	-	-	-	-	-	-	-
14	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1.5	-	-	10	-	-	-	-	-	-	-	-	-	-
16	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-
17	1.5	-	-	-	-	-	-	-	-	10,15	-	-	-	-
18	1.5	-	-	-	-	-	-	-	5	-	-	-	-	-
19	2.5	10	-	-	-	-	-	10	-	-	-	-	-	-
20	2.5	-	-	-	-	5	-	-	-	5,10,15	10,15	-	-	-
21	2.5	-	10	-	-	5,10,15	-	-	10	10,15	-	5	-	-
22	2.5	-	5	-	-	-	-	-	-	-	-	-	10,15	5
23	2.5	-	-	15	15	-	-	-	-	-	-	-	-	-
24	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 - Abnormal gastroscopy findings associated with horses given either intravenous sterile saline, or eltenac at 0.5 mg/kg, 1.5 mg/kg, or 2.5 mg/kg once daily for 15 days.

Horse	Saline or eltenac (mg/kg)	Day	Squamous Lesions (specified day)	Glandular Lesions (specified day)*
-	saline	-	no lesions seen	no lesions seen
7	0.5	12	no lesions seen	patchy reddening (Day 12)
9	0.5	-8, 12	linear erosion (Day -8), focal mucosal thickening and focal white discoloration (Day 12)	no lesions seen
10	0.5	7	2 pinpoint umbilicated erosions (Day 7)	no lesions seen
11	0.5	12	focal thickening of margo plicatus (Day 12)	no lesions seen
15	1.5	12	no lesions seen	scattered pinpoint red foci (Day 12)
17	1.5	-8, 12	focal thickening of margo plicatus (Day -8)	patchy reddening along rugal folds (Day 12)
18	1.5	12	no lesions seen	patchy reddening along rugal folds (Day 12)
20	2.5	12	focal thickening along margo plicatus and patchy reddening in small area (Day 12)	no lesions seen
21	2.5	7	no lesions seen	patchy reddening (Day 7)
22	2.5	-8,7,12	small round focal erosions (Day -8), scattered focal erosions (Day 7), scattered focal erosions	multiple linear erosions (Day 12)
24	2.5	-8,12	patchy red and white discoloration (Day -8)	patchy reddening along rugal folds (Day 12)

* Development of glandular lesions was significant (p=.02).

Table 3 - Results of hematology and coagulation parameters that were found to change significantly for horses given either intravenous sterile saline, or eltenac at 0.5 mg/kg, 1.5 mg/kg or 2.5 mg/kg once daily for 15 days (results shown are mean values/standard deviation).

Saline or eltenac (mg/kg)	Day	WBC (10^3 /ul) *	RBC (10^3/ul)	HGB (g/dl)	HCT (%)	Neutro-phils (10^3 /ul) *	APPT (seconds)	PT (seconds)	Fibrin-ogen (mg/dl)
saline	-1	11.8/3.5	8.5/2.0	12.4/3.1	37.9/10.1	6.8/2.4	45/6.2	10.9/.3	461/119
saline	3	10.0/2.0	8.3/1.7	12.1/2.9	37.0/9.6	5.1/.9	43/2.0	10.6/.2	595/82
saline	6	9.9/3.4	8.2/1.6	12.1/2.5	36.6/4.5	5.0/1.0	40/7.3	11.0/.3	594/70
saline	10	10.0/3.1	9.1/2.2	13.2/3.6	37.9/7.3	5.2/1.7	46/6.9	11.0/0	597/119
saline	15	9.4/2.3	8.5/1.5	12.4/2.5	33.7/4.6	4.0/1.8	44/8.2	10.2/.4	565/89
0.5	-1	9.2/3.0	8.8/1.0	12.6/1.7	37.9/4.9	3.9/1.9	60/7.7	10.4/.2	405/101
0.5	3	11.1/2.6	8.9/1.0	12.7/1.3	38.6/4.4	5.9/2.0	42/1.9	10.5/.3	609/69
0.5	6	10.1/2.1	8.2/0.5	11.7/0.9	35.7/3.2	5.2/2.3	44/4.6	10.4/.2	581/150
0.5	10	9.6/2.1	7.5/0.7	11.0/0.9	32.7/3.4	5.4/2.1	49/5.8	10.5/.2	512/97
0.5	15	9.6/1.8	7.9/0.6	11.3/0.4	34.1/1.7	4.5/1.6	45/6.9	10.0/.5	528/73
1.5	-1	11.1/3.3	9.4/1.2	13.4/1.1	41.7/4.0	5.7/2.1	46/5.1	10.5/.3	647/96
1.5	3	9.1/1.4	9.4/1.1	13.5/1.5	41.9/5.0	4.3/1.1	45/8.1	10.6/.5	554/42
1.5	6	7.8/1.9	9.5/1.1	13.7/1.5	42.4/5.3	3.3/1.1	47/4.4	10.6/.2	576/69
1.5	10	8.5/2.5	9.9/0.6	14.5/1.1	44.5/3.1	3.2/1.3	44/3.9	10.1/.5	558/84
1.5	15	10.7/2.5	8.8/1.2	12.8/1.9	39.5/6.0	5.4/2.1	42/4.5	9.2/.2	602/36
2.5	-1	11.4/1.9	9.1/0.9	13.0/1.6	39.9/5.5	6.0/2.1	50/6.1	11.0/.5	669/72
2.5	3	6.7/1.7	8.2/1.0	11.7/1.2	35.9/4.4	2.6/1.1	45/4.0	11.3/.4	649/91
2.5	6	7.2/1.8	8.3/0.7	12.0/1.4	36.6/4.5	1.7/.6	51/5.6	10.8/.5	597/58
2.5	10	12.5/3.5	8.6/1.2	12.4/2.0	37.9/7.3	6.2/3.3	40/5.7	10.2/.4	574/50
2.5	15	13.0/2.2	7.6/0.5	11.1/1.5	33.7/4.6	8.0/1.8	47/6.9	9.1/.6	583/120

* Indicates eltenac had a statistically significant dose-related effect on value

Table 4 - Mean biochemical values that were found to change significantly for horses given either intravenous saline or eltenac at 0.5 mg/kg, 1.5 mg/kg or 2.5 mg/kg once daily for 15 days (results shown are mean value/ standard deviation).

Saline or eltenac (mg/kg)	Day	Albumin (gm/dl) *	AST U/L	BUN (mg/dl)	Creatinine (mg/dl)	Globulin (gm/dl) *	GGT (U/L)	Mg (meq/L)	Osmolality (mos/kg)	TP (gm/dl) *	P (mg/dl)	Ca (mg/dl)	Cl (meQ/L)	Na (meQ/L)
saline	-1	3.2/.33	277/62	15/2.5	.95/.08	3.7/.68	9.8/3.0	1.6/.26	292/2.1	7.0/.4	2.5/.7	11.2/.6	103/2	141/1
saline	3	3.7/.45	285/60	16./2.4	1.1/.14	3.6/.75	9.8/2.7	2.0/.14	293/3.0	7.3/.3	2.6/.7	12.7/.5	106/1	141/2
saline	6	3.8/.44	271/52	19/3.0	1.1/.25	3.3/.82	8.8/2.4	1.8/.19	293/3.0	7.1/.5	2.8/.5	12.2/.3	103/1	141/1
saline	10	3.5/.39	272/49	21/3.1	1.1/.07	3.5/.58	10/3.2	1.8/.07	295/2.2	7.1/.2	3.7/1.1	12.1/.9	106/1	141/1
saline	15	3.4/.28	271/39	16/1.7	1.0/.10	3.1/.66	9.0/2.0	1.8/.12	294/3.8	6.6/.5	3.6/.7	11.6/.4	107/1	141/2
0.5	-1	3.6/.25	283/30	20/2.3	1.0/.12	3.2/.79	9.3/2.0	1.8/.15	295/3.3	6.8/.7	2.9/.6	12.1/.4	105/1	141/1
0.5	3	4.1/.42	314/27	20/2.5	1.2/.16	3.5/.71	10/2.2	2.0/.20	301/3.0	7.6/.6	3.0/.8	13.1/.3	107/2	144/1
0.5	6	4.0/.29	280/32	21/2.9	1.2/.18	2.8/.80	9.0/1.4	1.7/.16	294/1.4	6.8/.7	3.0/.3	12.1/.5	103/1	140/1
0.5	10	3.4/.16	289/29	24/2.9	1.3/.16	3.0/.70	9.8/1.1	1.5/.21	296/2.8	6.4/.7	3.2/.7	11.7/.4	107/2	140/1
0.5	15	3.5/.19	309/36	21/4.2	1.1/.17	2.9/.70	9.3/1.3	1.7/.14	297/4.1	6.4/.7	3.7/.6	11.6/.4	108/1	142/2
1.5	-1	3.7/.23	267/38	18/3.9	.95/.10	3.3/1.0	11/3.3	1.6/.34	292/3.1	7.0/1.0	2.4/.3	11.5/.7	103/1	140/1
1.5	3	3.8/.38	285/38	18/2.9	1.0/.13	3.5/.45	9.5/2.4	1.9/.23	293/1.5	7.4/.5	2.3/.6	12.8/.9	105/1	141/1
1.5	6	3.4/.37	278/42	24/4.0	.96/.29	3.4/.48	9.3/2.3	1.8/.17	295/3.9	6.8/.6	3.1/.5	11.6/.6	104/1	141/2
1.5	10	3.3/.33	277/39	21/2.4	1.2/.27	3.3/.86	9.1/1.4	1.6/.18	294/4.5	6.6/.7	2.4/.6	11.7/.6	109/1	141/2
1.5	15	2.7/.38	264/17	21/3.1	1.0/.13	2.9/.26	8.6/1.5	1.6/.18	289/3.2	5.7/.43	3.0/.4	11.0/.4	105/1	138/2
2.5	-1	3.4/.10	289/65	17/2.7	.91/.19	4.3/1.0	14/2.1	1.8/.15	293/6.3	7.2/.2	2.9/.5	11.6/.1	102/1	140/1
2.5	3	3.5/.08	28158	21/4.9	1.1/.25	2.8/1.2	13/2.7	1.7/.09	292/5.0	6.3/1.2	2.4/.5	12.2/.6	106/2	140/3
2.5	6	3.1/.32	240/44	21/2.6	1.2/.32	2.9/.25	10/2.0	1.5/.14	290/1.8	6.0/.3	3.1/1.1	10.9/.6	104/1	138/2
2.5	10	2.8/.35	268/70	20/2.4	1.3/.36	2.5/.33	10/2.5	1.5/.17	293/4.0	5.3/.6	3.1/1.1	10.9/.6	108/2	140/2
2.5	15	2.4/.4	226/67	19/4.1	1.1/.35	2.3/.28	9.5/2.4	1.5/.16	288/2.1	4.7/.6	3.2/.6	10.5/.4	105/1	138/1

* Indicates eltenac had a statistically significant dose-related effect on value

Table 5 - Post-mortem oral and stomach lesions in horses given etiher intravenous sterile saline or eltenac at .5 mg/kg, 1.5 mg/kg or 2.5 mg/kg once daily for 15 days. None of the changes were found to be related to drug dose (results shown as gross findings/histologic findings).

Horse	Saline or eltenac (mg/kg)	Oral Lesions	Stomach Nonglandular	Stomach Glandular
1	saline	NSF	NSF	NSF
2	saline	NSF	NSF	2 pinpoint lesions, 4x15mm/moderate focal erosion
3	saline	NSF	NSF	NSF
4	saline	NSF	NSF	NSF
5	saline	NSF	NSF	NSF
6	saline	NSF	NSF	NSF
7	0.5	NSF	NSF	11 linear lesions 2x20mm/minimal erosion
8	0.5	1cm diam. erosion on tongue/ erosion	NSF	NSF
9	0.5	NSF	NSF	NSF
10	0.5	NSF	NSF	NSF
11	0.5	NSF	NSF	NSF
12	0.5	NSF	NSF	NSF
13	1.5	NSF	multifocal erosion/minimal congestion	NSF
14	1.5	NSF	NSF	NSF
15	1.5	1.5x1cm erosion on ventral tongue/erosion	NSF	red areas/minimal lymphocytic infiltration
16	1.5	NSF	18, 3x18mm ulcerations/mod. ulceration	NSF
17	1.5	NSF	1, 3x4mm erosion/moderate erosion	NSF
18	1.5	NSF	NSF	5x15mm focal erosion/ erosion
19	2.5	small ulceration on labial surface of upper lip/ulceration	NSF	NSF
20	2.5	small focal erosion ventral tongue/erosion	11 linear erosions approx3-30mm/ erosion	NSF
21	2.5	NSF	NSF	15, 3x2mm ulcerations/erosion
22	2.5	NSF	NSF	multifocal erosion/mild erosion
23	2.5	5x3mm ulceration on upper lip/ulceration	NSF	NSF
24	2.5	NSF	NSF	reddened area/minimal congestion

NSF = no significant findings

Table 6 - Post-mortem intestinal lesions in horses given either intravenous sterile saline, or eltenac at .5 mg/kg, 1.5 mg/kg or 2.5 mg/kg once daily for 15 days. None of the changes were found to be related to drug dose (results shown as gross findings/histologic findings).

Horse	Saline or eltenac(mg/kg)	Small Intestine	Large Colon	Cecum	Small Colon
1	saline	serosal adhesion/adhesion	mucosal discoloration/moderate neutrophil infiltration	diffuse hyperemia/moderate hyperemia	NSF
2	saline	NSF	NSF	NSF	NSF
3	saline	NSF	NSF	NSF	NSF
4	saline	diffuse petechiation /minimal congestion	NSF	diffuse petechiation/ minimal hemorrhage	NSF
5	saline	NSF	NSF	NSF	NSF
6	saline	focal hyperemia/mild neutrophil infiltration	focal hyperemia/mild neutrophil infiltration	diffuse mucosal petechiation/moderate hemorrhage	NSF
7	0.5	NSF	NSF	NSF	NSF
8	0.5	NSF		NSF	NSF
9	0.5	serosal hemorrhage/ moderate congestion	NSF	NSF	NSF
10	0.5	yellow, fluid/no changes	NSF	NSF	NSF
11	0.5	petechiation/minimal congestion	petechiation/minimal congestion	NSF	NSF
12	0.5	NSF	NSF	NSF	NSF
13	1.5	red discoloration/mild congestion	NSF	NSF	NSF
14	1.5	NSF	NSF	NSF	NSF
15	1.5	congestion/mild congest.	NSF	NSF	NSF
16	1.5	NSF	diffuse petechiation/mild neutrophil infiltration	NSF	NSF
17	1.5	NSF	NSF	NSF	NSF
18	1.5	NSF	NSF	NSF	NSF
19	2.5	NSF	NSF	NSF	NSF
20	2.5	hyperemic/ mild congestion	NSF	NSF	NSF
21	2.5	hyperemia/mimal congestion	NSF	NSF	NSF
22	2.5	NSF	NSF	NSF	NSF
23	2.5	NSF	NSF	NSF	NSF
24	2.5	NSF	NSF	NSF	NSF

Table 7 - Post-mortem respiratory, renal and other gross findings in horses given either intravenous sterile saline or eltenac at .5 mg/kg, 1.5 mg/kg or 2.5 mg/kg once daily for 15 days. None of the changes were found to be related to drug dose (results shown as gross/histologic findings).

Horse	Saline or eltenac mg/kg	Respiratory	Renal	Other
1	saline	atelectsis, discoloration/pulmonary edema	NSF	left jugular thrombosis/inflammation,edema
2	saline	white foci/bronchiolitis	NSF	
3	saline	congestion/congestion	NSF	splenic congestion/same
4	saline	NSF	rt kidney - ecchymosis/no histologic changes	bladder-mucosal hyperemia/congestion
5	saline	NSF	NSF	NSF
6	saline	minimal congestion/congestion	NSF	bladder mucosal hyperemia/congestion
7	0.5	NSF	NSF	enlarged mesenteric lympnod/hyperplasia
8	0.5	consolidation/bronchopneumonia	NSF	esophagus ulcer in proximal third/ulcer
9	0.5	atelectasis/broncholitis	NSF	right jug thrombosis/inflammation
10	0.5	focal consolidation/ broncholitis	NSF	skin erosion around orbit/dermatitis
11	0.5	NSF	NSF	NSF
12	0.5	NSF	NSF	NSF
13	1.5	NSF	NSF	NSF
14	1.5	congestion in left lobe/ congestion	NSF	Pericardial adhesion/ no histo changes
15	1.5	NSF	NSF	
16	1.5	left lobe-congestion/congestion	NSF	bladder-mucosal hyperemia/congestion
17	1.5	NSF	NSF	NSF
18	1.5	serosal petechiation/ multifocal congestion	NSF	NSF
19	2.5	nodule 10cm in diameter/broncholitis	NSF	red pericardial fluid/no changes
20	2.5	NSF	NSF	left jugular swelling/ chronic inflammation
21	2.5	NSF	NSF	right jugular edema/ no changes
22	2.5	NSF	NSF	splenic adhesion/adhesion
23	2.5	white foci/minimal congestion	NSF	NSF
24	2.5	NSF	NSF	red pericardial fluid/no changes

Conclusion

Eltenac administered intravenously at dosages of 0.5mg/kg, 1.5mg/kg, and 2.5 mg/kg once daily did not cause any changes in physical examination findings. The common findings of moderate to severe oral, gastric and intestinal ulceration seen with commonly used NSAIDs did not occur in eltenac administered at 1, 3 and 5 times the intended therapeutic dose. Mild gastric ulcerations occured in the squamous and glandular mucosa of eltenac treated horses however the severity was minimal. A significant treatment effect was noted in the antemortem period for glandular ulcers however post-mortem examination did not reveal any signifance in treatment effect for mucosal lesions.

Significant drug related biochemical effects were those of neutropenia, hypoproteinemia, albuminemia and hypoglobulinemia. The white blood cell count rebounded during the second half of the 14 day period, however the panhypoproteinemia which was greatest for the high dose group worsened over the 15 day treatment period. The reason for these changes remains in question.

Post-mortem results did not reveal any significant drug related effects of eltenac given at the administered dosages. These findings

are important in assessment of judging the drug to be safe for a 15 day dosing regimen. The data from this study suggests that eltenac is a very safe NSAID.

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