

Effects of Nonsteroidal Anti-Inflammatory

Drug on Prevalence of Helicobacter Like Organisms in Gastric Mucosa of Thoroughbreds Horses

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Recibido: 20/01/2011

Aceptado: 20/03/2011

Abstract

The aim of this study was describe effects of Nonsteroidal Anti-inflammatory drug on prevalence of Helicobacter Like Organisms in gastric mucosa of Thoroughbreds horses. Were studied 54 Thoroughbred horses in the national race Track "La Rinconada" Caracas-Venezuela. All equine were treated by seven days with phenylbutazone at an intravenous dose of 4.4 mg/kg. All horses presented Equine gastric ulcer syndrome acute superficial gastritis (25/54), chronic gastritis with erosion focal (16/54), chronic gastritis with erosion focal and ulcers (14/54) in the gastric in both regions mucosa squamous region and glandular regions (fundus). Helicobacter Like Organisms infection in the stomach was confirmed by Warthin-Starry (38/54). Gastric mucosa revealed numerous spiral-shaped bacteria morphologically resembling Helicobacter Like Organisms in squamous regions, margo plicatus (20/38) and numerous spiral-shaped bacteria in fundic glands (18/54). In conclusion, we detected high presence of Helicobacter Like Organisms in the gastric mucosa of Thoroughbred horse's treatment with phenylbutazone.

Key words: Thoroughbred; horses; equine; HLO; Helicobacter; phenylbutazone; EGUS.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are substances other than steroids that inhibit a component of the inflammatory cascade¹. Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) are two well-known important causative factors of gastric injury such as gastritis and peptic ulcer. The interaction between these two factors in terms of their effects on gastric mucosa remains controversial². Helicobacter pylori (*H. pylori*) and non-steroidal anti-inflammatory drugs (NSAID) are major pathogenic factors in peptic ulcer disease but whether these two factors exert synergistic or antagonistic action on the gastric mucosa has been a subject of controversy³. There are many reasons for these controversies, including differences in the characteristics of subjects, differences in methods of approach, relatively small sample sizes, etc. Furthermore, the complicated mechanisms, including cyclooxygenase 2 (COX-2) and/or COX-1 activity, may be involved in the interaction between *H. pylori* and NSAIDs (nonselective and COX-2 selective) on the gastric mucosa. Therefore, an understanding of processes at the cellular level and large studies comparing COX-2 inhibitors with nonselective COX inhibitors will provide better information². Phenylbutazone (PBZ) is used as a non-steroidal anti-inflammatory drug (NSAID) for the treatment of chronic pain, including the symptoms of arthritis. The toxicity of phenylbutazone in the horse has been investigated very thoroughly in recent years and it has been shown to cause renotoxicity and, most significantly, ulceration of the gastrointestinal tract when relatively high doses are administered⁴. In Venezuela phenylbutazone is permitted in the racecourse of Thoroughbred horses. Side effects of phenylbutazone are similar to that of other NSAIDs. Overdose or prolonged use can cause gastrointestinal ulcers, blood dyscrasia, kidney damage, oral lesions, and internal haemorrhage, especially pronounced in young, ill, or stressed horses⁴. Helicobacter species have been detected and associated with equine gastric ulcer syndrome⁵. In Venezuela we detected the presence of Helicobacter-specific DNA in the

squamous and the glandular mucosae of Thoroughbred horses⁵. The aim of this study was describe effects of Nonsteroidal Anti-inflammatory drug on prevalence of Helicobacter Like Organisms in gastric mucosa of Thoroughbreds horses.

Material & methods

Animals: Were studied 54 Thoroughbred horses (30 female and 24 male), between 2-5 years old, in training in the national race Track "La Rinconada" Caracas-Venezuela.

Clinical signs: were lameness, acute abdominal pain recurrent and weight loss syndrome.

Therapeutically: All equine were treated by seven days with phenylbutazone at an intravenous dose of 4.4 mg/kg.

Necropsy and histology: All equine were euthanized and study by necropsy⁶. Samples of tissue were collected from the gastric mucosa, bowel kidneys and liver^{6,7}. The tissue samples fixed in formalin were processed by conventional histological techniques (dehydration, inclusion in paraffin, microtome slicing and routine staining with Hematoxylin-eosin). Additionally, the special staining procedure of Warthin-Starry, Toluidin blue and Giemsa was also carried out⁷.

Results

Necropsy: revealed weight loss, loss fatty subcutaneous, xantomatosis of subcutaneous tissue (20/54). Equine gastric ulcer syndrome severd in all horses, specifically acute superficial gastritis (25/54), chronic gastritis with erosion focal (16/54) (Figure 1a), chronic gastritis with erosion focal and ulcers (14/54) (Figure: 2a & 3a) in the gastric in both regions mucosa squamous region and glandular regions (fundus). Colitis chronic and focal hemorrhage (16/54).

Liver was swollen, friable with fibrosis chronic (36/54). Multifocal necrotic areas were present in the other lobes (45/50). Renal cortical and papillary necrosis, acute tubular necrosis (54/54).

Histology: The histologic slices revealed a loss of continuity of the gastric mucosa in 29/54 horses (Figures: 1b, 2b & 3b), with corium exposure and subcorionic edema with parakeratotic hyperkeratosis together with a mixed lymphoplasmocytic mononuclear infiltrate. With regard to ulcer distribution, both regions of the stomachs showed similar patterns of lesions. Included large numbers of lymphoid nodules throughout all regions of the gastric mucosa and were most numerous in the fundus and body. A mild, diffuse lymphocytic infiltrate with small numbers of plasma cells and eosinophils was also present in the subglandular region of all portions of the gastric mucosa. To determine now NSAIDs affect Helicobacter Like Organisms infection-induce of chronic inflammatory activity in the gastric mucosa were quantified by manual counting per field. The mononuclear cell score showed increasing in the presence of HLO infection. The neutrophil score was low. Chronic colitis lymphoplasmocitic (12/54). Liver with periaci-

nar necrosis with a prominent acinar pattern and fatty degeneration severd (45/50). Centre-acinar necrosis and bilirubin cluster (15/54). Necrosis and vacuolar (glycogen) degeneration islets of langerhans, fibrosis and chronic (12/54). Renal cortical and medullary necrosis, acute tubular necrosis, degeneration vacuolar and glycogen nephrosis (54/54), glomerulonephritis membranous (26/54).

Special staining: Helicobacter Like Organisms infection in the stomach was confirmed by Warthin-Starry (38/54) (Figures: 1c, 2c & 3c), Toluidin blue (35/54) and Giemsa staining (36/54). Gastric mucosa revealed numerous spiral-shaped bacteria morphologically resembling Helicobacter Like Organisms in squamous regions, margo plicatus (20/38) and numerous spiral-shaped bacteria in fundic glands (18/54).

Figures:

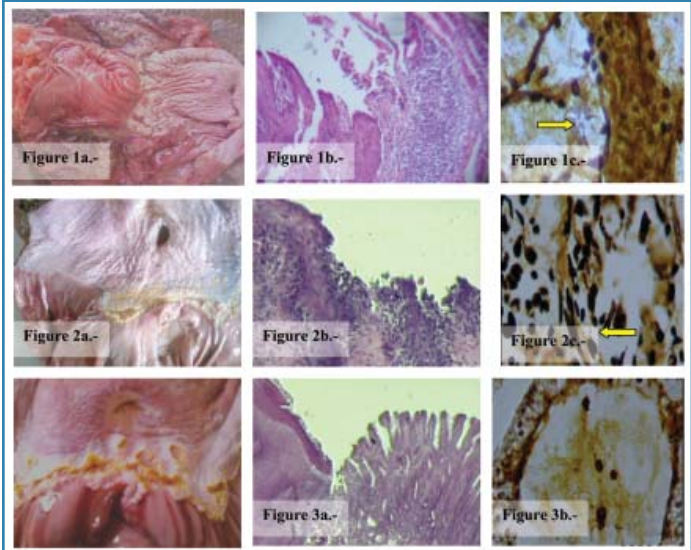


FIGURE 1a.- Stomach of equine with gastric ulcer syndrome. 1b.- Gastric mucosa with gastritis chronic erosion and ulcer with severd infiltrated of lymphocytes (Hematoxililn & Eosin 20X). 1c.- Gastric mucosa with special staining Warthing Starry positive with spiral shaped Helicobacter like organisms (arrow) (W&S 40X).

FIGURE 2a.- Stomach of equine with gastric ulcer syndrome. 2b.- Gastric mucosa with gastritis chronic focal ulcer with infiltrated of lymphocytes (Hematoxililn & Eosin 20X). 2c.- Gastric mucosa with special staining Warthing Starry positive with abundant spiral shaped Helicobacter like organisms in the fundic glands (arrow) (W&S 40X).

FIGURE 3.- Stomach of equine with gastric ulcer syndrome. 2b.- Gastric mucosa with gastritis chronic focal ulcer with infiltrated of lymphocytes (Hematoxililn & Eosin 20X). 3c.- Gastric mucosa with special staining Warthing Starry positive with spiral shaped Helicobacter like organisms in the fundic glands (arrow) (W&S 40X).

Discussion

Background Nonsteroidal anti-inflammatory drugs (NSAIDs) are some of the most widely prescribed drugs worldwide and have now probably overtaken H.pylori as the commonest cause of gastrointestinal injury in Western countries⁸. The use of NSAIDs is common in horses presenting with acute abdominal pain, lameneses and other pain. Typically, these horses are given either phenylbutazone or flunixin meglumine intravenously to control pain during a colic episode. Phenylbutazone and flunixin meglumine have been found to induce gastric ulcers in horses, but usually at higher-than-recommended doses. Several factors may predispose towards phenylbuta-

zone toxicity in the horse, including breed and age, but high dosage is considered to be particularly important⁴. NSAID are thought to cause more severe ulcers in the glandular mucosa because of their effect on prostaglandin inhibition⁹. Prostaglandins in inhibition results in decreased mucosal blood flow, decreased mucus production, and increased HCl secretion. Although prostaglandins are also important in the regulation of acid production and sodium transport, it may be their effect on mucosal blood flow that is the most important⁹. Gastric mucosal ischemia may lead to a hypoxia-induced cellular acidosis and release of oxygen-free radicals, phospholipase, and proteases, which may damage the cell membrane leading to necrosis. Although NSAIDs are commonly used, they have the potential to exacerbate EGUS in horses with colic. *Helicobacter* spp (other than *Helicobacter pylori*) have been isolated from humans and a variety of animals suffering from gastric ulcers and gastritis⁹. Furthermore, *Helicobacter*-like DNA was detected in the stomach of 10 Thoroughbred horses in Venezuela^{5,9}. In this study, *Helicobacter*-like DNA was detected in two of seven horses with gastric ulcers, three of five horses with gastritis, five of six horses with both pathologies, and one horse with normal gastric mucosa. Furthermore, 10 of 11 of the horses infected with *Helicobacter* had either gastric ulcers or gastritis or both pathologies. Bacterial colonization of gastric ulcers in the stomach of horses may delay ulcer healing⁹. *Helicobacter pylori* (*H. pylori*) infection and nonsteroidal anti-inflammatory drugs (NSAIDs) use are considered to be the most important risk factors having influence on the onset of bleeding gastroduodenal lesions¹⁰. The majority of the examined cases were associated with both *H. pylori* infection and NSAIDs use. A statistically significant difference among the studied groups of patients was proven. The majority of bleeding gastroduodenal lesions was associated with the coexistence of *H. pylori* infection and NSAIDs use, while their independent influences were statistically less important¹⁰. These results are humans and are similar to those observed in our study in horses. A high prevalence (70%) of ulcers and gastritis with *Helicobacter* Like Organisms and phenylbutazone was found in Thoroughbred racehorses during our study even though none of them had a previous record of gastrointestinal disorders at the time of their euthanasia. This agrees with other earlier studies reporting the occurrence of gastric ulcers in 80 to 90% of Thoroughbred racehorses^{11,12,13,14} and *Helicobacter* in horses 61%⁵. In a clinical report on the interaction between NSAIDs and *H. pylori*, Hawkey et al. showed that *H. pylori* eradication in long-term users of NSAIDs, with past or current peptic ulcer or troublesome dyspepsia, led to a impaired healing of the gastric ulcers², implying that under certain circumstances some patients with *H. pylori* are less prone to NSAID-induced ulceration than noninfected patients. This may be due to the opposing effects of *H. pylori* and NSAIDs on PG synthesis in the gastric mucosa². However, other studies have shown that the eradication of *H. pylori* prior to NSAID therapy reduces the occurrence of peptic ulcers², and the level of apoptosis in gastric mucosae, and that NSAID users infected with *H. pylori* carry a greater risk of peptic ul-

cer than noninfected NSAID users². It was also reported that *H. pylori* infection may reduce the adaptation threshold and that the eradication of *H. pylori* restored the ability of the gastric mucosa to adapt to aspirin². In the equine large intestine, exogenous prostaglandins had a variable effect on contractile activity, depending on the location in the colon and orientation of the smooth muscle. The administration of NSAID inhibited contractility, with flunixin meglumine generally inducing the most profound inhibition relative to the other NSAID evaluated in substance P-stimulated smooth muscle of the large intestine. The results of this study indicate that prolonged use of NSAID may potentially predispose horses to develop gastrointestinal tract stasis and subsequent impaction¹⁵. In rats with preexisting chronic gastric ulcers, *H. pylori* infection attenuated significantly the aspirin-induced inhibition of ulcer healing and accompanying fall in the gastric blood flow at the margin of these ulcers, suggesting negative interaction between aspirin and *H. pylori* on ulcerogenesis. Accumulated evidence in humans and animals shows that both aspirin and *H. pylori* upregulate the expression of cyclooxygenase (COX)-2 both at mRNA and protein levels at the ulcer margin, but failed to influence significantly that of COX-1. It was, therefore, proposed that *H. pylori* may in fact, antagonize, aspirin-induced delay of ulcer healing due to suppression of acid secretion by the enhancement in PGE² possibly derived from COX-2 expression and activity and to the overexpression of growth factors such as TGF alpha and VEGF³. Lesions in the liver and kidney toxicity suggest NSAIDs in all horses studied. The lesions of the colon were not significant, which differs with reports in the literature^{4,11,12,13,14}.

Conclusion

In conclusion, we detected high presence of *Helicobacter* Like Organisms in the gastric mucosa of Thoroughbred horse's treatment with phenylbutazone. In reviewing current knowledge of the clinical pharmacology of this important group of drugs, it is hoped to provide the clinician with a rational, scientific basis for their safe and effective use in equine practice.

Acknowledgments

This study was funded by the PG 11-00-6631-06 CDCH Central University of Venezuela.

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