Effects of Nonsteroidal Anti-Inflammatory

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

Abelardo Morales Briceño, Francisco Garcia, Victor Bermúdez García
Department of Veterinary Pathology Faculty of Veterinary Science Central University of Venezuela Maracay, Aragua State Venezuela.

Corresponds author: Abelardo Morales Department of Veterinary Pathology Faculty of Veterinary Science, Central University of Venezuela, Aragua State. Building 3, office 3, Campus Maracay.
Email: aamorales13@gmail.com Phone: 058 0243 550 60 29 Telefax: 058 0243 550 60 29 Cell phone: 058 0414 4563101

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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 subs
es other than steroids that inhibit a component of the in
matory cascade[1]. Helicobacter pylori and nonsteroidal
inflammatory drugs (NSAIDs) are two well-known impo
causative factors of gastric injury such as gastritis and p
ulcer. The interaction between these two factors in ter
their effects on gastric mucosa remains controversial[2].
cobacter pylori (H. pylori) and non-steroidal anti-inflamm
drugs (NSAID) are major pathogenic factors in peptic
disease but whether these two factors exert synergistic o
tagonistic action on the gastric mucosa has been a su
of controversy[3]. There are many reasons for these contr
sies, including differences in the characteristics of sub
differences in methods of approach, relatively small sa
sizes, etc. Furthermore, the complicated mechanisms, in
ing cyclooxygenase 2 (COX-2) and/or COX-1 activity,
be involved in the interaction between H. pylori and NS
(nonselective and COX-2 selective) on the gastric mu
Therefore, an understanding of processes at the cellular
and large studies comparing COX-2 inhibitors with nons
tive COX inhibitors will provide better information[2]. Phe
utazone (PBZ) is used as a non-steroidal anti-inflamm
drug (NSAID) for the treatment of chronic pain, includin
symptoms of arthritis. The toxicity of phenylbutazone i
horse has been investigated very thoroughly in recent y
and it has been shown to cause renotoxicity and, most si
cantly, ulceration of the gastrointestinal tract when rela
high doses are administered[4]. In Venezuela phenylbuta
is permitted in the racecourse of Thoroughbred horses.
effects of phenylbutazone are similar to that of other NSA
Overdose or prolonged use can cause gastrointestinal ul
blood dyscrasia, kidney damage, oral lesions, and int
haemorrhage, especially pronounced in young, ill, or stre
horses4. Helicobacter species have been detected an
sociated with equine gastric ulcer syndrome[5]. In Venez
we detected the presence of Helicobacter-specific DNA i

squamous and the glandular mucosae of Thoroughbred horses[5]. 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 necropsy[6]. 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, Toloudin blue and Giemsa was also carried out[7].

Results

Necropsy: revealed weight loss, loss fatty subcutaneous, xantomathosis of subcutaneous tissue (20/54). Equine gastric ulcer syndrome severed 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 expossure 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 periacinar necrosis with a prominent acinar pattern and fatty de
eration severed (45/50). Centre-acinar necrosis and billi
cluster (15/54). Necrosis and vacuolar (glycogen) dege
tion islets of langerhans, fibrosis and chronic (12/54). F
cortical and medullary necrosis, acute tubular necrosis
generation vacuolar and glycogen nephrosis (54/54), g
erulonephritis membranous (26/54).

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

Figures:

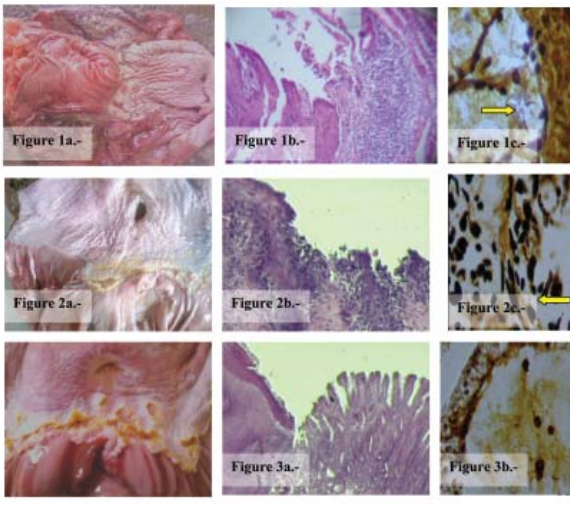

FIGURE 1a.- Stomach of equine with gastric ulcer syndrome. 1b.- Gastric
with gastritis chronic erosion and ulcer with severed infiltrated of lymphocyt
matoxilin & Eosin 20X). 1c.- Gastric mucosa with special staining Warthing
positive with spiral shaped Helicobacter like organisms (arrow) (W&S 40X).

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

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

Discussion

Background Nonsteroidal anti-inflammatory drugs (NSA
are some of the most widely prescribed drugs worldwide
have now probably overtaken H.pylori as the comm
cause of gastrointestinal injury in Western countries[8]. Th
of NSAIDs is common in horses presenting with acute ab
inal pain, lameneses and other pain. Typically, these h
are given either phenylbutazone or flunixin meglumine
venously to control pain during a colic episode. Phenyl
zone and flunixin meglumine have been found to induce
tric ulcers in horses, but usually at higher-than-recomme
doses. Several factors may predispose towards phenyl

zone toxicity in the horse, including breed and age, but high dosage is considered to be particularly important[4]. NSAID are thought to cause more severe ulcers in the glandular mucosa because of their effect on prostaglandin inhibition[9]. 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 important[9]. 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 gastritis[9]. 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 healing[9]. 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 lesions[10]. 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 coexistance of H. pylori infection and NSAIDs use, while their independent influences were statistically less important[10]. 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 phenylbutazona 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 ocurrence 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 ulcers[2], implying that under certain circunstances 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 mucosa[2]. However, other studies have shown that the eradication of H. pylori prior to NSAID therapy reduces the occurrence of peptic ulcers[2], and the level of apoptosis in gastric mucosae, and that NSAID users infected with H. pylori carry a greater risk of peptic ulcer than noninfected NSAID users[2]. It was also reporte
H. pylori infection may reduce the adaptation threshol
that the eradication of H. pylori restored the ability of the
tric mucosa to adapt to aspirin[2]. In the equine large inte
exogenous prostaglandins had a variable effect on contr
activity, depending on the location in the colon and orient
of the smooth muscle. The administration of NSAID inhi
contractility, with flunixin meglumine generally inducin
most profound inhibition relative to the other NSAID e
ated in substance P-stimulated smooth muscle of the
intestine. The results of this study indicate that prolonge
of NSAID may potentially predispose horses to develop
trointestinal tract stasis and subsequent impaction[15]. In
with preexisting chronic gastric ulcers, H. pylori infectio
tenuated significantly the aspirin-induced inhibition of
healing and accompanying fall in the gastric blood flow a
margin of these ulcers, suggesting negative interactio
tween aspirin and H. pylori on ulcerogenesis. Accumu
evidence in humans and animals shows that both aspiri
H. pylori upregulate the expression of cyclooxygenase (C
2 both at mRNA and protein levels at the ulcer margin
failed to influence significantly that of COX-1. It was, t
fore, proposed that H. pylori may in fact, antagonize, as
induced delay of ulcer healing due to suppression of aci
cretion by the enhancement in PGE^2 possibly derived
COX-2 expression and activity and to the overexpressi
growth factors such as TGF alpha and VEGF[3]. Lesions i
liver and kidney toxicity suggest NSAIDs in all horses stu
The lesions of the colon were not significant, which c
with reports in the literature[4,11,12,13,14].

Conclusion

In conclusion, we detected high presence of Helicobacte
Organisms in the gastric mucosa of Thoroughbred ho
treatment with phenylbutazone. In reviewing current k
edge of the clinical pharmacology of this important gro
drugs, it is hoped to provide the clinician with a rational, s
tific basis for their safe and effective use in equine pract

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References

1. Moses, V.S., Bertone, A.L. (2002). Nonsteroidal anti-inflamm
drugs. Vet Clin North Am Equine Pract. 18, 21-37.

2. Tae Il Kim,1,* Yong Chan Lee,1 Kwang Hyoung Lee, et al. (200
fects of Nonsteroidal Anti-Inflammatory Drugs on Helicobacter
Infected Gastric Mucosae of Mice: Apoptosis, Cell Proliferatio
Inflammatory Activity. American Society for Microbiology. 69,
5063.

3. Brzozowski, T., Konturek, P.C., Sliwowski, Z., et al. (2006). Inter
of nonsteroidal anti-inflammatory drugs (NSAID) with Helico
pylori in the stomach of humans and experimental animals. J F
Pharmacol. 57, 67-79.

4. Lees, P., Higgins, A.J. (1985). Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse. Equine Vet J. 17, 83-96.

5. Contreras, M., Morales, A., Garcia-Amado, M., et al. (2007). Detection of Helicobacter-like DNA in the gastric mucosa of Thoroughbred horses. Letters in Applied Microbiology. 45, 553-557.

6. Aluja, A., Constantino, C. (2002). Technical of Necropsy in domestic animals. Manual Moderno. México.

7. Banks, W. (1996). Veterinary Applied Histology. Manual Moderno México.

8. Musumba, C., Pritchard, D.M., Pirmohamed, M. (2009). Review article: Cellular and molecular mechanisms of NSAID-induced peptic ulceration. Aliment Pharmacol Ther. 3, 222.

9. Videla, R., Andrews, F. (2009). New Perspectives in Equine Gastric Ulcer Syndrome. Veterinary clinics of North America 25, 287-288.

10. Golubovi□, G., Tomasevi□, R., Radojevi□ B., et al. (2007). Significance of Helicobacter pylori infection and nonsteroidal anti-inflammatory drugs in patients with bleeding from upper part of the gastrointestinal tract]. Vojnosanit Pregl.64, 8-445.

11. Murray, M.J., Schusser, G.F., Pipers, F.S., et al., (1996). Factors associated with gastric lesions in thoroughbred racehorses. Equine Vet J. 28, 368-374.

12. Pagan, J.D. (1999). Gastric ulcers in horses: a widespread but manageable disease. Equinews. 2, 16-17.

13. Jones, W.E. (2002). Understanding gastric ulcers in horses. J Equine Vet Sci. 22, 330.

14. Andrews, F.M., Buchanan, B.R., Elliot, S.B. et al. (2005). Gastric ulcers in horses. J Anim Sci. 83, 18-21.

15. Van Hoogmoed, L.M., Snyder, J.R., Harmon, F. (2000). In vitro investigation of the effect of prostaglandins and nonsteroidal anti-inflammatory drugs on contractile activity of the equine smooth muscle of the dorsal colon, ventral colon, and pelvic flexure. Am J Vet Res. 61, 66-1259.