Comparison of gastric adverse effects of aspirin and celecoxib before and after eradicating *Helicobacter* spp. infection in dogs

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Summary

The present study was carried out to determine whether *Helicobacter* spp. infection in dogs could affect lesions caused by the non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and celecoxib. Thirty asymptomatic mixed-breed dogs were used for this study. Gastric biopsies were taken via gastroscopy and evaluated by polymerase chain reaction (PCR) and rapid urease tests (RUT). *Helicobacter* spp. was detected in all specimens. The infection was completely eradicated in 15 dogs by a three-drug regimen for 21 days (amoxicillin, clarithromycin and omeprazole). Each *Helicobacter*-positive and -negative group was then divided into three subgroups treated with aspirin, celecoxib and placebo for 14 days. Dogs in the different subgroups were compared by sequential gastroscopy on days 0, 3, 7, 14 and 21 (a week after drug cessation). The results show that selective COX-2 inhibitors are better tolerated than conventional non-selective NSAIDs in dogs in terms of their side effects; however, these drugs should be administered with caution. Unlike what is advised in medical practices in humans, it seems that the eradication of non-*pylori Helicobacter* spp. from the stomach is not necessary prior to administrating NSAIDs in dogs.

Key words: Aspirin, Celecoxib, *Helicobacter*, PCR, Dog

Introduction

Cyclooxygenase (COX) is a rate-limiting enzyme that catalyzes the conversion of arachidonic acid into prostaglandins (PGs) and prostanoids. Cyclooxygenase has two distinct membrane-anchored isoenzymes, COX-1 and COX-2. The former is constitutively expressed and found in most normal body tissues, while the latter is expressed in normal tissues at low levels and is highly induced by pro-inflammatory mediators in inflammation, injury, and pain settings (Radi and Khan, 2006).

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory, analgesic and anti-pyretic effects by reducing the synthesis of prostaglandins via COX inhibition. However, because they suppress COX-1 and COX-2 simultaneously, one of their main side effects is gastric lesions (Nishihara *et al*., 2001).

During the past decade, a new generation of NSAIDs that selectively block the COX-2 has been developed. Examples are drugs such as meloxicam, carprofen, celecoxib and etodolac. These agents are expected to have lower gastric side effects (Laine, 2003). However, using them in combination with corticosteroids may still be ulcerogenic (Simpson, 2010).

On the other hand, a high prevalence of GHLOs (gastric *Helicobacter*-like organisms) (67 to 100%) is reported in gastric mucosa of dogs in several countries, including Iran (Buczolits *et al*., 2003; Shabestari *et al*., 2008; Simpson, 2010). Despite the controversy surrounding this issue, GHLOs seem to contribute to the production of gastric disorders such as chronic gastritis and gastric erosion/ulcers (Harbour and Sutton, 2008; Simpson, 2010). There are also reports that show ameliorations of gastric disorders after the eradication of *Helicobacter* spp. in dogs (Happonen *et al*., 2000; Leib *et al*., 2007).

Despite the high frequency of GHLOs in dogs and cats and the widespread usage of NSAIDs (as pain killers or anti-inflammatory medication) in small animal medicine, little is still known about their concurrent effects on gastric mucosa.

Generally, it seems that *Helicobacter* infection and NSAID use can each cause gastric erosion/ulcer; however, they also have a synergic interaction in producing gastropathies (Barkin, 1998). Consequently, to decrease the occurrence of gastric side effects of NSAIDs, it seems reasonable to eliminate *Helicobacter* spp. from the stomach before prescribing these drugs. On the other hand, there are also reports revealing that *Helicobacter* spp. can have inhibitory effects on NSAID related complications by increasing Prostaglandin E2 (PGE2) in the gastric mucosa (Fiorucci *et al*., 1999). For this reason, the present study was carried out to determine if *Helicobacter* spp. infection can affect lesions caused by NSAIDs (aspirin as a conventional non-selective NSAID and celecoxib as a selective COX-

2 inhibitor) in dogs.

Materials and Methods

Animals and sampling procedure

This study was approved by the Iranian laboratory animal ethics framework under the supervision of the Iranian Society for the Prevention of Cruelty to Animals. A total of 30 mixed-breed dogs (mean age 1.5 years) were selected from different locations of Shiraz, Iran. The animals were observed for seven days, during which their health status was confirmed by clinical and laboratory examinations. The 30 asymptomatic healthy dogs underwent 12 h of fasting and were premedicated with intramuscular injections of Acepromazine maleate (Castran, Interchemie, Holland) (0.05 mg/kg) and xylazine hydrochloride (Alfasan, Woerden, Holland) (0.5 mg/kg). They were then anesthetized with a combination of diazepam (Phoenix Pharma Ltd., Gloucester, England) (0.25 mg/dog), and ketamine hydrochloride (Alfasan, Woerden, Holland) (5-10 mg/kg). Gastroscopy was then performed with a 7.9 mm diameter gastroduodenoscope (MEDIT/Canada) to obtain two pairs of biopsies from their bodies and antral regions of the stomach in order to detect *Helicobacter* spp. using polymerase chain reactions (PCR) and rapid urease tests (RUT).

DNA extraction and PCR amplification

DNA was extracted from gastric biopsy specimens using a DNeasy tissue kit (Qiagen, Germany) according to the manufacturers' instructions. PCR amplifications were performed in a final volume of 25 µl containing 100 ng extracted DNA, 2.5 µl 10 x PCR buffer (Fermentas, Lithuania), 0.2 mM dNTP, 1.5 mM $MgCl_2$, 25 pmol/µl of each primer and 0.2 U Taq DNA polymerase (Fermentas, Lithuania). The PCR was carried out using a MJ-Mini BioRad thermal cycler (BioRad, USA) with an initial denaturing cycle at 94°C for 4 min, followed by 33 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min. A final extension step was then carried out at 72°C for 7 min (Shojaee Tabrizi *et al*., 2010). The resulting PCR products underwent gel electrophoresis (1.0% agarose gel with ethidium bromide (0.5 mg/l) and were visualized under a UV transilluminator. The primer sequence used in this study for the detection of *Helicobacter* spp. was (F): 5´-AAGG ATGAAGCTTCTAGCTTGCTA-3´, (R): 5´-GTGCTTA TTCGTGAGATACCGTCAT-3´ (Shojaee Tabrizi *et al*., 2010). The size of the expected fragment (398 bp) was compared to a 100 bp reference marker (Fermentas).

Rapid urease test (RUT)

A pair of gastric biopsy specimens was placed into urea broth media (DIFCO, USA) and incubated at 37ºC for 24 h. Color transformation from yellow to pink/red within 24 h was considered as a positive result with the following degrees: color change within the first 2 h (+3), between 2 and 6 h (+2) and between 6 and 24 h (+1). No color transformation within 24 h was considered negative (0) (Ricci *et al*., 2007).

Drug administration

All 30 dogs with proved *Helicobacter* spp. infection (by PCR and RUT) were randomly divided into two groups. Of these, 15 dogs considered as the *Helicobacter* spp. negative group underwent a therapeutic protocol with amoxicillin (Cosar-amoxicillin, Tehran, Iran; 20 mg/kg, twice a day), clarithromycin (Claricin, Tehran Shimi, Iran; 7.5 mg/kg, twice a day) and omeprazole (Kharazmi, Tehran, Iran; 0.5-1 mg/kg, once a day) for three weeks. After this treatment period, *Helicobacter* infection status was again assessed (by PCR and RUT) in the manner described previously. Each of the mentioned groups was further divided into 3 subgroups according to Table 1.

Consequently, gastroscopy was performed on days 0, 3, 7 and 14 of the treatment, and day 21, a week after drug cessation. Before each endoscopy, all equipment and instruments were cleaned thoroughly and sterilized in 2% glutaraldehyde (Behsadex, Behsa Pharmaceutical, Arak, Iran) for at least 20 min, and rinsed completely with normal saline. Multiple gastric biopsies were obtained by endoscopy on day 0 to determine each dog's *Helicobacter* infection status. All regions of the stomach were evaluated and assigned scores from 0 to 6 for each endoscopic evaluation as follows (Murtaugh *et al*., 1993):

0: No visible hemorrhage, erosion or ulcers
1: 1-5 punctate erosions and/or hemorrhages
2: 6-15 punctate erosions and/or hemorrhages
3: 16-25 punctate erosions and/or hemorrhages
4: ≥25 punctate erosions and/or hemorrhages or 1-5 invasive erosions
5: ≥5 invasive erosions
6: Ulcers of any sizes

Statistical analysis

Obtained scores by the subgroups within each group were compared separately for each day using a Kruskal-Wallis test. If any significant difference was seen, a Mann-Whitney U test was run to determine the different subgroups. In addition, to compare the effects of the

Table 1: Experimental groups according to gastric *Helicobacter* spp. status and drug administration

Groups	Placebo	Aspirin	Celecoxib
Helicobacter spp. positive	Subgroup 1	Subgroup 2	Subgroup 3
Helicobacter spp. negative	Subgroup 4	Subgroup 5	Subgroup 6

Subgroups 1 and 4 received placebo p.o. q12h, subgroups 2 and 5 received 25 mg/kg aspirin (ASA, Pars Darou Industrial Co., Tehran, Iran) p.o. q12h, and subgroups 3 and 6 received 3 mg/kg celecoxib (celexib, Chemi Darou Industrial Co., Tehran, Iran) p.o. q12h. All treatments continued for 14 consecutive days

drugs between *Helicobacter*-positive and -negative groups, a Mann-Whitney U test was used. To evaluate the effects of NSAID administration on each group, a repeated measure ANOVA was run.

Results

Helicobacter infection status

Genus-specific PCR identified 30/30 (100%) of the subjects as *Helicobacter* spp.-positive in the gastric mucosa (Fig. 1). Rapid urease tests confirmed these results. After three weeks of anti-*Helicobacter* therapy for 15 dogs, PCR and RUT results showed the complete eradication of *Helicobacter* spp. from gastric mucosa (Fig. 2).

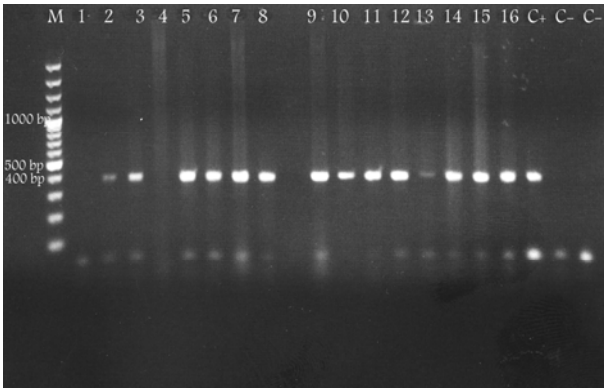


Fig. 1: PCR amplification of DNA extracted from biopsy samples by species-specific primers of *Helicobacter* genus before treatment. Lane M: 100 bp molecular ladder, Lanes 1-16: Biopsy samples, Lane C+: Positive control, and Lane C-: Negative control

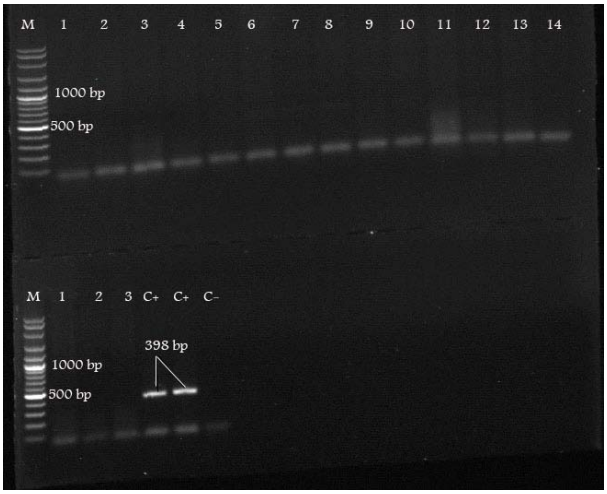
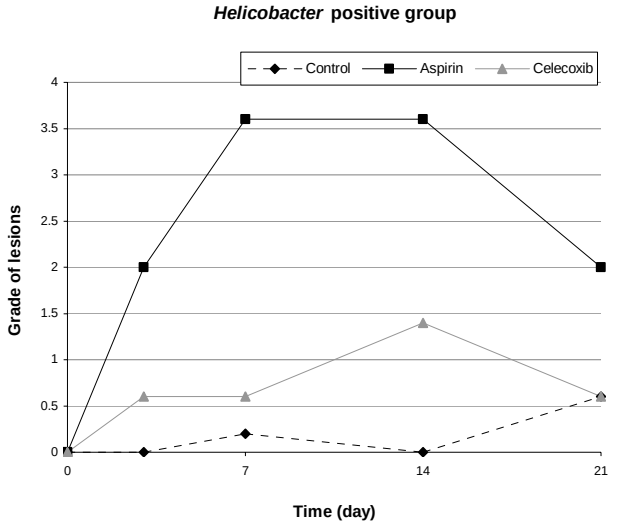


Fig. 2: PCR amplification of DNA extracted from biopsy samples by species-specific primers of *Helicobacter* genus after treatment. (up) Lane M: 100 bp molecular ladder, Lanes 1-14: Biopsy samples, (down) Lane M: 100 bp molecular ladder, Lanes 1-3: Biopsy samples, Lane C+: Positive contro, and Lane C-: Negative control

Endoscopic evaluation

The results of endoscopic evaluations of both *Helicobacter*-positive and -negative groups are shown in Figs. 3a, b. Although the COX-2 inhibitor drug, celecoxib, produced some erosions in both *Helicobacter* spp.-positive and -negative groups, there was no significant difference among dogs that received celecoxib compared to those who were given the placebo ($P>0.05$). Stomach lesions in dogs that received aspirin were significantly more severe than those receiving celecoxib or the placebo in both *Helicobacter*-positive and -negative groups ($P<0.05$). However, our results showed that gastric *Helicobacter* infection status in dogs had no significant effect on celecoxib and aspirin treatment consequences ($P>0.05$).

a



b

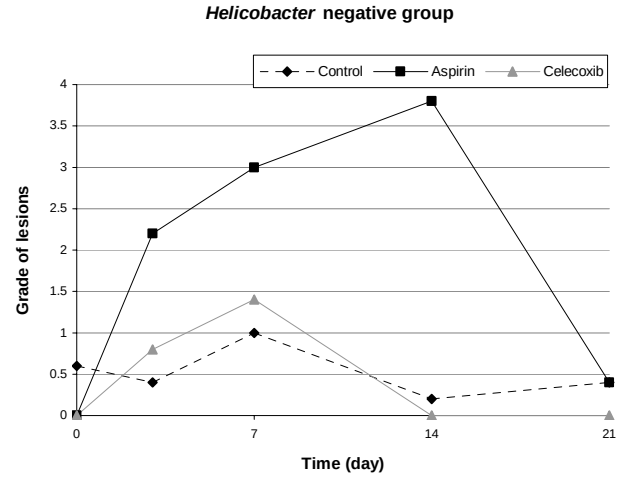


Fig. 3: Gastric lesion score in subgroups of a) *Helicobacter*-positive (n=15), and b) *Helicobacter*-negative dogs (n=15)

Discussion

In the current study, a volcanic ulcer was observed in one of the *Helicobacter* spp.-infected dogs in the celecoxib group (day 14), but no significant difference was detected between gastric lesions caused by celecoxib compared to the placebo, supporting the results of previous studies (Nishihara *et al*., 2001). Although, COX-2 selective NSAIDs seem to cause minus gastric

lesions in comparison to conventional non-selective NSAIDs, this study showed that the administration of celecoxib in dogs must be done with caution and the risk of their consequences should not be neglected.

While our study revealed no significant differences in the production of gastric mucosal injuries between the *Helicobacter* spp.-positive and -negative dogs in either aspirin or celecoxib treatment groups, human studies show beneficial effects of *H. pylori* eradication on preventing gastropathies induced by NSAIDs (Bazzoli *et al*., 2001). In another study on rats, an antagonistic relationship was reported between *H. pylori* and aspirin in the creation of gastric lesions (Konturek *et al*., 2002). Although, species-specific PCR was not performed in the present research, based on other studies, almost all of the isolates from canine gastric mucosa were non-*pylori Helicobacter* species, including *H. heilmannii* and *H. felis* (Harbour and Sutton, 2008; Shabestari *et al*., 2009; Simpson, 2010). According to the results of the present study, neither non-*pylori Helicobacter* infections nor the administration of NSAIDs (conventional non-selective or COX-2 selective NSAIDs) acted synergistically or antagonistically in producing gastric erosion/ulcers. Such controversy between different study designs might be due to the specific characteristics of the hosts and various kinds of *Helicobacter* spp. Nevertheless, for more accurate interpretation, more studies will be required.

Our study also showed that gastric lesions observed in aspirin subgroups, both in *Helicobacter* spp.-positive and -negative dogs, were significantly more severe than the placebo group. Many reports in the literature support this result (Shiotani *et al*., 2008; Shiotani *et al*., 2009; Kim *et al*., 2012). In our study, gastric lesions were first seen on day 3 of aspirin administration to both *Helicobacter* spp.-positive and -negative groups, and the severity of lesions increased concomitantly with the progression of the treatment until day 14 (the last day of drug administration) decreasing later on day 21 (Figs. 3a, b). Nishihara *et al*. (2001) reported that the gastric lesions started on day 14, the severity of which gradually increased. Despite the fact that in our study, drug administration dosage and interval were the same as Nishahara *et al*. (2001), the cause of such differences is not clearly known, although they might be attributed to a number of environmental factors such as feeding schedules, types of food and husbandry (Thirlby and Feldman, 1984). In spite of this, both studies showed significant correlations between treatment durations and lesion severity.

It is concluded that for dogs, the side effects of selective COX-2 inhibitors are better tolerated than those of conventional non-selective NSAIDs; however, they should be administered with caution. Unlike what is advised in human medicine, it seems that the eradication of non-pylori *Helicobacter* spp. from the stomach is not necessary prior to the administration of NSAIDs in dogs.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Barkin, J (1998). The relation between *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs. Am. J. Med., 105: 22-27.

Bazzoli, F; De Luca, L and Graham, DY (2001). *Helicobacter pylori* infection and the use of NSAIDs. Best Pract. Res. Cl. Ga., 15: 775-785.

Buczolits, S; Hirt, R; Rosengarten, R and Busse, HJ (2003). PCR-based genetic evidence for occurrence of *Helicobacter pylori* and novel *Helicobacter* species in the canine gastric mucosa. Vet. Microbiol., 95: 259-270.

Fiorucci, S; Antonelli, E; Santucci, L; Morelli, O; Miglietti, M; Federici, B; Mannucci, R; Soldato, PD and Morelli, A (1999). Gastrointestinal safety of nitric oxide-derived aspirin is related to inhibition of ICE-like cysteine proteases in rats. Gastroenterology. 116: 1089-1106.

Happonen, I; Linden, J and Westermarck, E (2000). Effect of triple therapy on eradication of canine gastric Helicobacters and gastric disease. J. Small Anim. Pract., 41: 1-6.

Harbour, S and Sutton, P (2008). Immunogenicity and pathogenicity of *Helicobacter* infections of veterinary animals. Vet. Immunol. Immunopathol., 122: 191-203.

Kim, Y; Yokoyama, S; Watari, J; Hori, K; Yamasaki, T; Okugawa, T; Toyoshima, F; Kondo, T; Sakurai, J and Tanaka, J (2012). Endoscopic and clinical features of gastric ulcers in Japanese patients with or without *Helicobacter pylori* infection who were using NSAIDs or low-dose aspirin. J. Gastroenterol., 47: 1-8.

Konturek, PC; Brzozowski, T; Kwiecien, S; Drozdowicz, D; Harsch, IA; Meixner, H; Stachura, J; Hahn, EG and Konturek, SJ (2002). Effect of *Helicobacter pylori* on delay in ulcer healing induced by aspirin in rats. Eur. J. Pharmacol., 451: 191-202.

Laine, L (2003). Gastrointestinal effects of NSAIDs and coxibs. J. Pain Symptom Manage., 25: 32-40.

Leib, MS; Duncan, RB and Ward, DL (2007). Triple antimicrobial therapy and acid suppression in dogs with chronic vomiting and gastric *Helicobacter* spp. J. Vet. Intern. Med., 21: 1185-1192.

Murtaugh, RJ; Matz, ME; Labato, MA and Boudrieau, RJ (1993). Use of synthetic prostaglandin E1 (misoprostol) for prevention of aspirin-induced gastroduodenal ulceration in arthritic dogs. J. Am. Vet. Med. Assoc., 202: 251-256.

Nishihara, K; Kikuchi, H; Kanno, T; Tanabe, S; Sarashina, T and Uzuka, Y (2001). Comparison of the upper gastrointestinal effects of etodolac and aspirin in healthy dogs. J. Vet. Med. Sci., 63: 1131-1133.

Radi, ZA and Khan, NK (2006). Effects of cyclooxygenase inhibition on the gastrointestinal tract. Exp. Toxicol. Pathol., 58: 163-173.

Ricci, C; Holton, J and Vaira, D (2007). Diagnosis of *Helicobacter pylori*: invasive and non-invasive tests. Best Pract. Res. Cl. Ga., 21: 299-313.

Shabestari Asl, A; Jamshidi, Sh; Mohammadi, M; Soroush, MH; Bahadori, A and Oghalaie, A (2009). Assessment of antimicrobial resistance of cultivable *Helicobacter*-like organisms in asymptomatic dogs. Iranian J. Vet. Res., 10: 241-249.

Shabestari, AS; Mohammadi, M; Jamshidi, S; Sasani, F; Bahadori, A and Oghalaie, A (2008). Assessment of chronic gastritis in pet dogs and its relation with *Helicobacter*-like organisms. Pakistan J. Biol. Sci., 11: 1443-1448.

Shiotani, A; Kamada, T and Haruma, K (2008). Low-dose aspirin-induced gastrointestinal diseases: past, present, and future. J. Gastroenterol., 43: 581-588.

Shiotani, A; Sakakibara, T; Yamanaka, Y; Imamura, H; Tarumi, KI; Manabe, N; Kamada, T; Kusunoki, H; Hata, J and Haruma, K (2009). Upper gastrointestinal ulcer in Japanese patients taking low-dose aspirin. J. Gastroenterol., 44: 126-131.

Shojaee Tabrizi, A; Jamshidi, S; Oghalaei, A; Zahraei Salehi, T; Bayati Eshkaftaki, A and Mohammadi, M (2010). Identification of *Helicobacter* spp. in oral secretions vs. gastric mucosa of stray cats. Vet. Microbiol., 140: 142-146.

Simpson, KW (2010). Diseases of the stomach. In: Ettinger, SJ and Feldman, EC (Eds.), *Textbook of veterinary internal medicine: diseases of the dog and cat*. (7th Edn.), St. Louis, Saunders Elsevier Inc., PP: 1504-1526.

Thirlby, RC and Feldman, M (1984). Effect of chronic sham feeding on maximal gastric acid secretion in the dog. J. Clin. Invest., 73: 566-569.