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 administering 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-
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 MgCl₂, 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 (Shojae 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 ATGAAGCTTCTAGCCTGCTA-3', (R): 5'-GTGCTTA TTCGTCGAGATACCGTACAT-3' (Shojae 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 37°C 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 (Behsaderex, 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 (Murtagh 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

Observed 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

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<th>Table 1: Experimental groups according to gastric Helicobacter spp. status and drug administration</th>
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<td>Groups</td>
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<tr>
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<td>Helicobacter spp. positive</td>
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<td>Helicobacter spp. negative</td>
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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](image1)

![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 control, and Lane C: Negative control](image2)

**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).

![Fig. 3: Gastric lesion score in subgroups of a) *Helicobacter*-positive (n=15), and b) *Helicobacter*-negative dogs (n=15)](image3)

**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. helimannii 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.

Acknowledgement

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