

Effects of licofelone, a novel 5-LOX inhibitor, in comparison to celecoxib on gastric mucosa of dogs

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Abstract

Despite the extensive application of non-steroidal anti-inflammatory drugs (NSAIDs), the use of these drugs is limited due to their adverse effects especially on gastric mucosa. Dual inhibitors that inhibit both cyclooxygenase (COX) and lipoxygenase (LOX) metabolites are considered to have less gastric toxicity in comparison to non-selective and COX-2 selective inhibitors. In this study, fifteen mixed breed dogs were randomly divided into three groups: group 1 (n=5) received placebo, group 2 (n=5) licofelone, an inhibitor of COX-1, COX-2, and 5-LOX (2.5 mg/kg; twice daily) and group 3 (n=5) celecoxib, a COX-2 selective inhibitor (3 mg/kg; twice daily) *per os* for 14 days. All dogs underwent blinded gastroscopies on days 0, 7, 14 and one week after cessation of treatment and gastric lesions were scored. Examinations to detect fecal occult blood were performed daily. Results showed that licofelone is significantly better tolerated than celecoxib in terms of gastric side effects (P=0.008). Therefore, it seems that licofelone can be an appropriate alternative in dogs when NSAID therapy is necessary. Occult blood was not detected in any dog during the study.

Introduction

Non-opioid analgesics, especially non-steroidal anti-inflammatory drugs (NSAIDs), are one of the most frequent medications used in small animal medicine. Most NSAIDs exert their anti-inflammatory, analgesic and anti-pyretic effects by reducing the synthesis of prostaglandins (PGs) via cyclooxygenase (COX) inhibition. COX has two distinct membrane-anchored isoenzymes, COX-1 and COX-2. The former is constitutively expressed and found in normal body tissues, while the latter is expressed in most of the normal tissues at low levels and is highly induced by pro-inflam-

matory mediators in inflammation, injury, and pain settings.¹ Although COX-2 specific drugs such as COXIBs (celecoxib, deracoxib, rofecoxib) have less gastric side effects than non-selective ones. Reports show that they are not as safe as they are expected to be.²

Arachidonic acid (AA) is a substance that is converted to PGs and leukotriens (LTs) by COX and 5-lipoxygenase (LOX) enzymes, respectively. LTs are responsible for inflammations and NSAIDs-induced gastrointestinal damages. Studies showed that diminishing leukotriene B4 levels in gastric mucosa will result in gastroprotection against NSAIDs-induced gastropathy.³ The inhibition of COX enzyme may lead to a shunt of AA metabolism towards 5-LOX pathway, and therefore, treatment with NSAIDs increase the formation of LTs possibly leading to gastric damage.⁴ Thus, the idea of dual inhibition *i.e.* COX/5-LOX inhibition emerged as an alternative and safe therapy for enhanced analgesic and anti-inflammatory effect with little or no gastric mucosal damage.⁵

Recently, a new generation of anti-inflammatory drugs, licofelone, has been introduced with the ability of simultaneous inhibition of prostaglandins, leukotrienes and thromboxanes. The promising advantage of this drug is its higher efficacy in controlling inflammation and lower gastric adverse effects. For this reason, the present study was carried out to determine if celecoxib can be replaced by this new drug in canine medicine.

Materials and Methods

Animals

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 15 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. All dogs were fed with chicken skeleton during the study.

Drug administration

All 15 dogs were randomly divided into three groups: group 1 (n=5) received encapsulated methylcellulose (placebo) twice daily, group 2 (n=5) received encapsulated licofelone (Merckle GmbH, Ulm, Germany) 2.5 mg/kg twice daily and group 3 (n=5) celecoxib (Celexib, Darou Pakhsh, Iran) 3 mg/kg, twice daily *per os* for 14 days.

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Endoscopy procedure

All dogs underwent 12 hours 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) on days 0, 7, 14 and 21 to obtain a thorough examination of gastric mucosa. The examiner (Shojaee Tabrizi) was blinded to the nature of treatment. After 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. All observations were recorded on tape for scoring the lesions. All regions of the stomach were

evaluated and assigned scores from 0 to 7 for each endoscopic evaluation as presented in Table 1.⁶

Statistical analysis

A non-parametric Kruskal-Wallis test at $P < 0.05$ was used to investigate whether the score of gastric lesions of three groups differed or not. The differences in gastric lesions between two groups were investigated by the Mann-Whitney U-test. Since this was multiple testing of the data the significance level was adjusted by using the Bonferroni test. Three groups were compared and therefore the significance level became 0.05 divided by 3; ($P = 0.017$).

Results

On the beginning of the study (day 0), mild gastric lesions were observed in two celecoxib-treated dogs and in four licofelone-treated dogs. No lesions were observed in the placebo group at either the baseline or the final (day 21) endoscopy. Table 2 shows the median and quartiles of the score of gastric lesions for each group on days 0, 7, 14 and 21.

After one week, following 7 days of continuous treatment, there were significant differences between the groups in terms of their endoscopic lesion scores for stomach. Celecoxib-treated dogs had more severe gastric mucosal damage in comparison to placebo and licofelone-treated dogs ($P = 0.008$). After two weeks (day 14), same results were observed in different groups and scores of gastric lesions in celecoxib-treated dogs were still significantly higher than the two other groups ($P = 0.008$). Interestingly, licofelone-treated dogs had no significant difference with placebo-treated dogs during the study period ($P > 0.017$). Two celecoxib-treated dogs that had pre-existing lesions (susceptible dogs) showed progression in their pre-existing gastric lesions on day 7 and one of them showed even more progression on day 14. In contrast, three of the four dogs that had gastric mucosal damage at baseline and treated with licofelone (susceptible dogs) experienced a regression in

their lesions, which was reflected by a lower sum in their gastric lesion scores. One of them had steady score over the treatment period.

One week after cessation of treatment (day 21), results show that score of gastric lesions was not significantly different between groups at $P < 0.017$. Indeed, gastric lesions of celecoxib-group and licofelone-group improved after one week of discontinuing of treatment.

No abnormalities in physical or fecal examinations, including fecal occult blood, were detected during the course of the study.

Discussion and Conclusions

The aim of this study was assessing the side effects of one of the COXIB class of NSAIDs, celecoxib, in small animal practice and comparison of it with one of the new generation of this class of drug. In present study, some of the dogs had pre-existing lesions on day 0, before starting the study, which is a common finding in dogs because of non-*pylori Helicobacter* infections, stress and foreign body ingestion. We did not exclude them from the study because most of the dogs in practice have the same lesions.⁶

Licofelone was administered at a dose of 2.5 mg/kg twice daily based on previous studies.^{6,7} In this study, licofelone had obviously a better safety profile in comparison to celecoxib. Same results were previously obtained in another study that compared rofecoxib with licofelone

in dogs⁶ and it seems that licofelone is better tolerated than COXIB class of NSAIDs. Also, results propose that leukotriene suppression *via* licofelone has more effective protection on gastric mucosa than prostaglandin inhibition occurring through selective or non-selective COX-2 actions, the fact that was previously mentioned in the study conducted by Moreau and colleagues.

Using selective or non-selective COX-2 inhibitors apparently cause an imbalance that shift the pathways to 5-LOX, consequently diverting arachidonic acid to overproduction of the noxious and chemo attractant leukotrienes.^{6,8-10} These events may justify the reason why a 5-LOX inhibitor like licofelone is much better tolerated than selective and non-selective COX-2 inhibitors.

Since in the present study no dog had abnormal clinical signs or positive result of fecal occult blood examination, our findings are in line with the results of other studies^{6,11} about the fact that dogs with gastric lesions may not show any clinical or laboratory sign of gastric damage caused by NSAIDs. Therefore, clinician should be alert that the risk of severe complications remains, even without any clinical sign.

Our study suggests the replacement of COXIBs and non-selective NSAIDs with a 5-LOX inhibitor, licofelone because of its excellent safety profile. However, performing more studies in regard with the efficacy of licofelone in managing the inflammatory and painful process in dogs is strongly recommended.

Table 1. System used for the scoring of gastric lesions.

0	Normal mucous membrane
1	1–10 petechiae
2	>10 petechiae
3	1–5 erosions (an erosion being defined as a lesion producing a definitive discontinuation of the mucous membrane, but without depth)
4	6–10 erosions
5	11–25 erosions
6	>25 erosions
7	One ulcer (an ulcer being defined as a lesion of variable size, but with a depth that is unequivocal)

Table 2. Median and quartiles of the score of gastric lesions for each group on days 0, 7, 14 and 21.

Groups	N	Day 0			Day 7			Day 14			Day 21		
		Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3
Control	5	0	0	0	0	0	0	0	0	0	0	0	0
Celecoxib	5	0	0	1	2	3	4	3	4	5	0.5	1	2
Licofelone	5	0.5	1	1.5	0.5	1	1	0	0	0.5	0	0	0.5

N, number of dogs; Q, quartiles.

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