

Effect of selected non-steroidal anti-inflammatory drugs on the pathomorphology of the mucous membrane of the canine colon

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ABSTRACT: Non-steroidal anti-inflammatory drugs (NSAIDs) have been commonly used for the management of chronic pain caused by inflammatory joint disease in dogs. Although effective at relieving pain and inflammation, NSAIDs are associated with a significant risk of serious gastrointestinal side effects. The present study was therefore designed to investigate the effects of carprofen as a poorly-selective COX (cyclooxygenase) inhibitor and robenacoxib as a selective COX-2 inhibitor on the colon mucosa. A biopsy of the gastrointestinal tract was performed before treatment and on the last day of treatment with orally-administered carprofen (Group I), robenacoxib (Group II) and empty gelatine capsule (Group III) for twenty-one days in a randomised study. The most evident microscopic lesions in the colonic mucosa in young beagles were caused by a 21-day treatment with robenacoxib. The infiltration with inflammatory cells in the lamina propria of the colonic mucosa was the most commonly-found histopathological lesion.

Keywords: carprofen; robenacoxib; cyclooxygenase; beagle; gastrointestinal tract; inflammatory cells

List of abbreviations

ARI = absolute risk increase, BM = body mass, CBC = complete blood count, COX = cyclooxygenase, GI = gastrointestinal, IC₅₀ = half maximal inhibitory concentration, NSAIDs = non-steroidal anti-inflammatory drugs, PGE₂ = prostaglandin, PGI₂ = prostacyclin

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of chronic pain caused by arthritis in dogs. They have potent analgesic and anti-inflammatory activity, inhibit transmission in the nociceptors and are indicated for prevention and treatment of joint oedema and inflammation, especially in the musculoskeletal system (Lascelles et al. 2005, 2007). NSAIDs are one of the best medicaments to prevent and treat post-surgical pain in dogs (Mathews 2000). A search for novel non-steroidal anti-inflammatory drugs has been simultaneously pursued in two directions: (1) towards finding compounds with more potent anti-inflammatory activity and (2) substances with less-severe adverse effects (Miedzybrodzki 2004; Bjorkamn 2006). All NSAIDs have a similar mechanism of activity, which consists in inhibiting the

activity of cyclooxygenase – an enzyme which participates in one of the pathways of arachidonic acid transformation (Sakamoto et al. 2006; Lascelles et al. 2007). Cyclooxygenase has at least three isoforms. COX-1 has been described as a constitutive isozyme (a so-called “good” isozyme) whose final products may be involved in physiological processes (protective effects on the gastrointestinal tract, maintenance of correct kidney function, regulation of homeostasis) (Clark et al. 2003; Deneuche et al. 2004), whereas COX-2 has been described as an inducible isozyme (a so-called “bad” isozyme) whose final products take part only in pathological processes (such as pain, inflammation, fever, inhibition of apoptosis) (Dow et al. 1990; Esteve et al. 2005). COX-3 is a recently-discovered COX isoform whose role in the inflammatory reaction has

not yet been fully elucidated (Giraudel et al. 2005, 2009). It is clear that COX-2 inhibitors will be safer than the classic NSAIDs that inhibit both COX-1 and COX-2 (Hazenwinkel et al. 2003; Almansori et al. 2005). Unfortunately, these assumptions have been found to be partially erroneous.

Long-term administration of NSAIDs (both traditional and coxibs) is always associated with adverse effects. A literature review has revealed a lack of data on the comparison between the impact of carprofen and robenacoxib on the colonic mucosa in dogs (Jones et al. 1992; Kubiak et al. 2004). Human studies have indicated an increasing incidence of adverse effects on this section of the GI (gastrointestinal) tract and a correlation with the administration of coxibs (Lanas et al. 2003; Fujimori et al. 2008, 2010). However, the number of similar studies carried out in dogs is limited (Lascelles et al. 2005; Lanas et al. 2009).

The present paper discusses the impact of carprofen and robenacoxib on the gross and microscopic morphology of the colonic mucosa in dogs.

MATERIAL AND METHODS

Animals. The study was carried out with 15 healthy Beagle dogs aged 13–14 weeks. The puppies were placed in facilities designed for laboratory animals and were fed a complete canine diet, i.e., Puppy Junior Diet with fish Forza 10 (Forza, Italy). Water was provided *ad libitum*.

Experimental model. The experiment was conducted in accordance with the recommendations specified by The Local Ethics Commission and The International Council for Laboratory Animal Science and The Council of Europe (The Resolution of the Local Ethics Commission No 15/2011 of March, 30 2011 and the Individual Permit No 1/2011 for the experiment with animals issued on 11 March 2011 by the Dean of the Faculty of Veterinary Medicine, UWM in Olsztyn) (Brylinska and Kwiatkowska 1996).

The dogs were randomly divided into three groups ($n = 5$). For three weeks, the animals were orally administered: Group I – carprofen (Rimadyl®; Pfizer Poland) at 2 mg/kg BM (body mass); Group II – robenacoxib (Onsior™, Novartis, UK) at 2 mg/kg BM; and Group III (control) – an empty gelatine capsule. In the first stage of the experiment (before the introduction of treatment), CBC (complete blood count) and blood chemistry, a parasitologi-

cal faecal examination and a test for faecal occult blood were performed (Szweda et al. 2012). Further, samples of colonic mucosa were collected for histopathological analysis. Endoscopy combined with sampling of the mucosa from the descending colon was repeated after 21 days of treatment. A physical examination was performed on a daily basis. Except for a few cases of diarrhoea and haematochezia, no unusual clinical symptoms were recorded in animals in any of the examined groups.

An anaesthesiologist applied a similar procedure for all cases of colonoscopy. Premedication was introduced with atropine sulphate (Atropinum sulfuricum, Polfa, Poland) at 0.05 mg/kg BM. Induction for general anaesthesia was performed with a combination of xylazine (Rometar, Spofa, Czech Republic) at 1 mg/kg BM and ketamine (Bioketan, Biovet, Poland) at 4 mg/kg BM administered simultaneously in one syringe into the cephalic vein. Following cessation of the laryngeal reflex, all dogs were intubated with a laryngoscope (Heine, Germany). Anaesthesia was maintained with isoflurane (Isoflurane, Abbot, UK) at a concentration of 1.8–2%.

Biopsy of the colonic mucosa was performed with a gastrofiberscope Olympus GIF XQ-30 (Olympus, USA) with a 1030 mm probe, an external collar diameter of 9.8 mm and a canal diameter of 2.8 mm. The diameter of the working canal of approx. 3 mm allowed for introduction of 2.4 × 1500 mm endoscopic biopsy forceps equipped with two oval biopsy spoons with a window. During the biopsy material was sampled for histopathological analysis. The veterinarian who performed the biopsy was blind to the drugs which were administered to the dogs.

The collected specimens of the mucosa were fixed in 10% neutralized formalin. In the next stage, the tissues were saturated with the so-called “intermediate” solutions and embedded in paraffin blocks. The microtome sections were stained with haematoxylin and eosin (Jacobs et al. 1990; Kleinschmidt et al. 2006). The preparations were then examined histopathologically under a Nikon Eclipse 80i optical microscope with a Nikon PS-Fi1 digital camera.

The microscopic lesions in the colon (infiltration with lymphoid cells into the *lamina propria*, increase in the gland diameter, proliferation of the connective tissue, hyperaemia) were evaluated according to the following scale: 0 = a lack of pathology in the image of healthy tissue, 1 = minor lesions, 2 = moderate lesions, and 3 = severe lesions.

Statistical analysis. The statistical analysis of histopathological lesions was performed using the Q Cochran test, chi-squared statistics and by calculating the absolute risk increase (ARI). The Q Cochran test allowed for a comparison of the changes of the actual status of colonic mucosa before and after treatment. For the purpose of the test, the microscopic lesions were encrypted in the following way: a situation in which there was no histopathological lesion before and after treatment was encoded as “0”; if a lesion was found both at the beginning and at the end of the experiment – then it was also denoted as “0”; if a lesion was absent before treatment but was present after 21 days – this was denoted as “1”; if at the beginning of the experiment a lesion was evaluated as one and on day 21 it was assessed at 2 or 3 – then it was attributed the code “1”.

RESULTS

Based on the CBC and blood chemistry, parasitological faecal examination and a test for faecal occult blood and colonoscopic examination, all dogs were classified as healthy upon commencement of the study (Szweda et al. 2012). Microscopic examination did not reveal any morphological lesions in the colonic mucosa in these dogs.

Among the microscopic lesions observed on day 21 of the experiment, infiltration with inflammatory cells was most frequently detected (62% of subjects) (Figure 1). Circulatory disturbances were the second most common group of morphologi-

cal lesions (49% of individuals). Hyperaemia and extravasation were also reported (Figure 2). In 43% of dogs, the diameter of glands in the colonic mucosa was increased and their lumen was filled with a substantial amount of mucus and desquamated epithelial cells. The lesions were each time assessed as mild (Figure 3). Among the observed morphological lesions in the examined section of the gastrointestinal tract, proliferation of the connective tissue organised in thick bands located between the crypts was the least frequent pathology (Figure 4); this lesion was confirmed in only 31% of the examined biopsy specimens.

The Q Cochran test for the colon revealed some statistically significant differences between the groups of dogs ($P < 0.001$). The highest number of pathologically changed morphological pictures was detected in the colonic mucosa of the dogs administered robenacoxib (75%) in comparison with the carprofen group (60%), whereas in the control group the examined microscopic picture was evaluated as “0” in all dogs.

The χ^2 statistical analysis was used to compare the severity of individual histopathological lesions in the colonic mucosa between the groups of dogs. The most intense infiltration with lymphoid cells in the lamina propria of the colon was reported in the dogs from Group II. This type of lesion was detected in all dogs from this group: in four of them it was mild and in one dog it was moderate. The findings in the dogs administered carprofen were comparable (mild lesions were detected in four individuals).

The result of a χ^2 test for the variable describing the increase in the diameter of glands in the colonic

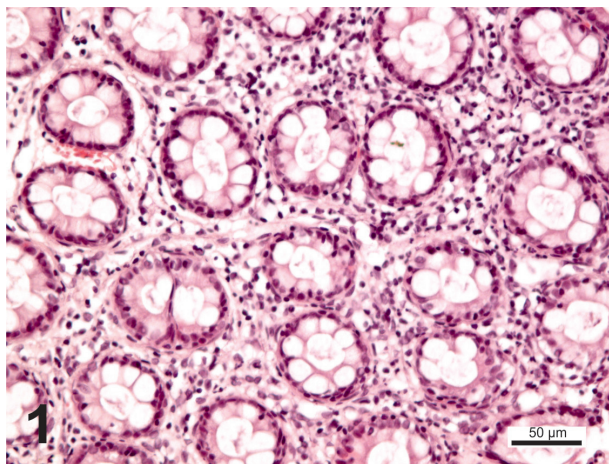


Figure 1. Infiltration with inflammatory cells in the lamina propria of the colon between the glands in a dog administered robenacoxib for 21 days; HE staining

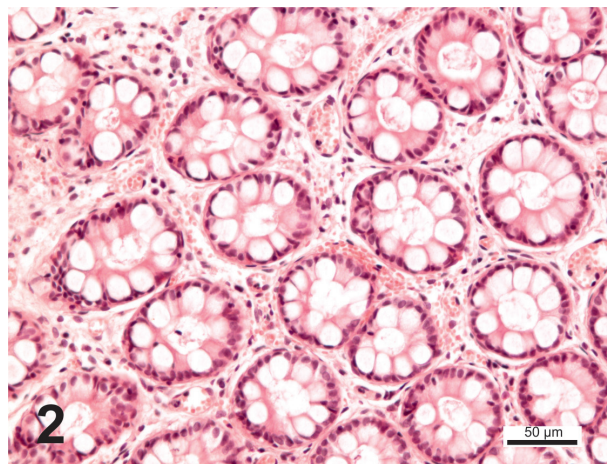


Figure 2. Hyperaemia of the colonic mucosa with focal extravasation in a dog administered robenacoxib for 21 days; HE staining

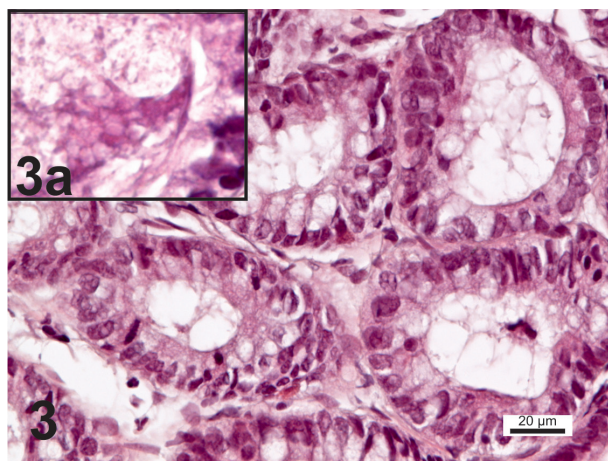


Figure 3. Enlarged glands with lumen filled with a substantial amount of mucus and desquamated epithelial cells in a dog administered carprofen for 21 days. **3a.** Pattern of the fragment gland with these lesions in 60× magnification; HE staining

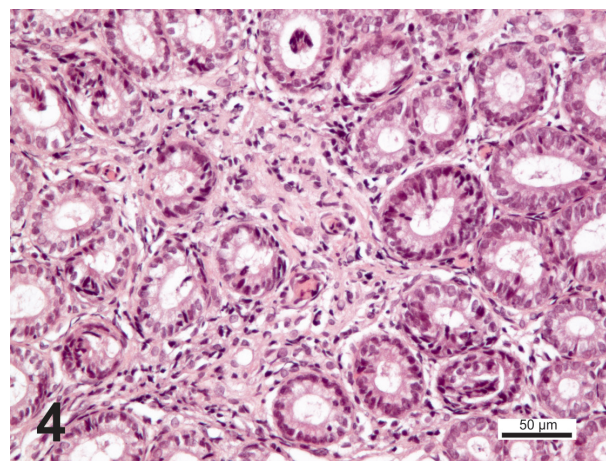


Figure 4. Proliferation of the fibrous tissue arranged in thick bands located between the crypts. The lumen of glands filled with mucus and dead epithelial cells. A dog administered carprofen for 21 days; HE staining

mucosa confirmed that a pathological lesion was detected in the majority of dogs administered robenacoxib (the lesions were reported in four dogs). In the biopsy specimens collected from the dogs administered carprofen for 21 days, an increase in the diameter of glands in the colonic mucosa was observed. Analogical findings were recorded for hyperaemia of the colonic mucosa and they were characterised as mild.

Mild proliferation of the connective tissue was detected in the biopsy specimens obtained from two dogs which were administered carprofen. It should be noted that in the animals receiving robenacoxib the findings were more advanced and severe (one individual with mild lesions and two individuals with moderate lesions).

Due to the fact that the results of the χ^2 test for low numerosness should be interpreted with caution, in further stages of the analyses the absolute risk increase (ARI) was calculated and its confidence intervals were depicted. This measurement is independent of the number of cases and it is calculated as the difference in the risk for the occurrence of a pathological lesion before and after the intervention. For the purpose of the test, the microscopic lesions were encrypted in the same way as for the Q Cochran test.

The risk for development of histopathological lesions in the colon increased most dramatically in Group II (ARI = –0.75), whereas a better result was reported in the group administered carprofen for 21 days (Group I – ARI = –0.6).

Table 1. Comparison between COX-1 and COX-2 expression in the gastrointestinal tract in different species (Radi and Khan 2006)

Gastrointestinal tract	COX-1					COX-2				
	Dog	Horse	Human	Monkey	Rat	Dog	Horse	Human	Monkey	Rat
Stomach fundus	X		X	X	X					
Pyloric antrum	X		X	X	X					
Duodenum	X		X	X	X					
Jejunum	X	X	X	X	X		X			
Ileum	X		X	X	X					
Cecum	X		X	X	X					X
Colon						X		X	X	

X = expression cyclooxygenase described

DISCUSSION

The experiments reported here allowed us to compare the impact of two NSAIDs on the colonic mucosa. These drugs, despite comparable therapeutic activity, differ in their degree of selective cyclooxygenase inhibition and should, therefore, provoke differing severities of adverse effects. Carprofen is a non-steroidal anti-inflammatory drug and an aryl derivative of propanoic acid that has potent anti-inflammatory, analgesic and antipyretic activity. It is a preferential COX-2 inhibitor. The ratio of selective inhibition of COX-1 to COX-2 declared by the manufacturer (Rimadyl[®], tablets for dogs) is at least 1 : 3 (Ricketts et al. 1998; Kay-Mugford et al. 2000; Wilson et al. 2004).

Robenacoxib is a novel compound belonging to the coxibs (a subclass of non-steroidal anti-inflammatory drugs) which have been introduced to the market. It is claimed that they are suitable for the treatment of pain and inflammatory conditions in dogs and cats (King et al. 2009, 2010). The pharmacological profile of a coxib shows a high degree of selective COX-2 inhibition (King et al. 2010), a short half-life in the blood and a longer-lasting effect at the site of inflammation (King et al. 2009). A summary of the product characteristics for Onsior tablets for dogs[®] specifies that in *in vivo* blood tests robenacoxib was found to be 140 times more selective for COX-2 (half maximal inhibitory concentration IC_{50} 0.004 μ M) than to COX – 1 (IC_{50} 7.9 μ M) (King et al. 2010; Silber et al. 2010).

The frequency at which a given drug damages the gastrointestinal mucosa depends on several factors, of which PGE_2 (prostaglandin) and PGI_2 (prostacyclin) inhibition is the most important. Carprofen shows a significantly higher degree of COX-1 inhibition than robenacoxib and, therefore, more potently blocks the biosynthesis of PGE_2 as well as prostacyclin (Jones et al. 1992; Lascelles et al. 1998; Kay-Mugford et al. 2000; King et al. 2011).

However, the results this study indicate more severe damage to the colonic mucosa with the administration of robenacoxib to young beagles. These findings may apply to the whole population, but they need to be further confirmed in studies carried out with larger groups of animals. These results run counter to the assumption that selective NSAIDs have a wider safety profile in comparison with preferential inhibitors. It seems probable that such a result is associated with the activation of an arachidonic acid transformation pathway

(lipooxygenase pathway) due to COX-2 inhibition. In addition, leukotrienes (as the final products of this pathway) further damage the gastrointestinal mucosa (Wooten et al. 2010) and, thus, even the NSAIDs with higher affinity to COX-2 also induce some damage to the GI tract.

Gretzer et al. showed that the application of selective COX-2 inhibitors to rats caused more severe damage to the gastrointestinal mucosa after the later administration of irritant substances (Gretzer et al. 2001). Similarly, ischemia of the gastric mucosa in rats was increased with the administration of highly selective COX-2 inhibitors (Wallace et al. 1990). This phenomenon could be best explained by the restoration of blood flow by means of the production of prostaglandins that induce vasodilatation. It has been shown that COX-2 plays a significant role in healing GI ulcers (Brzozowski et al. 2001). Lanas et al. have shown that in the case of large bowel inflammation, COX-2 inhibition resulted in exacerbation of an inflammatory condition, leading to perforation and death (Lanas et al. 2003). It should be noted that King et al. reported that carprofen shows weak COX inhibition when administered in therapeutic doses, which may explain the relatively low incidence of adverse effects associated with this drug (King et al. 2010).

Expression of COX-1 and COX-2 in the gastrointestinal tract varies among species and is presented in Table 1 (Radi and Khan 2006). Both COX-1 expression and relative COX-1/COX-2 are higher in some animal species (including dogs and rats) in comparison with humans, and this fact may partially explain the sensitivity of these species to even low NSAID doses (Table 1) (Wilson et al. 2004; Radi and Khan 2006). COX-2 expression in the colon in the dog has attracted special attention.

The unfavourable effects of robenacoxib may be related not only to cyclooxygenase inhibition, but also to the direct toxic impact of the drug on the colonic mucosa (Warner et al. 1999; Lichtenberger et al. 2012).

CONCLUSIONS

In the present study, we demonstrated that carprofen induced a significant and positive effect on the colonic epithelium compared to robenacoxib in young beagle dogs. Therefore, when considering a treatment with NSAID in these animals, it would be more beneficial to use carprofen.

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