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EFFECT OF MELOXICAM AND MELOXICAM WITH MISOPROSTOL ON SERUM PROSTAGLANDINS AND GASTROINTESTINAL PERMEABILITY IN HEALTHY BEAGLE DOGS

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The aim of the present study was to investigate the effect of meloxicam and meloxicam with misoprostol on prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) serum concentration, as well as on gastrointestinal permeability. NSAIDs, such as meloxicam, have gastrointestinal side effects, which are due to prostaglandins depletion and topical damage. Seven adult beagle dogs were included in the study. Three different 20 days long treatments were carried out (placebo, meloxicam and meloxicam with misoprostol). The same seven dogs participated in all three treatments. On days 1 to 10 the dogs received placebo, meloxicam or meloxicam together with misoprostol PO. Dogs were than monitored from day 11 to 20. Samples for serum PGE_2 and PGI_2 concentration and plasma lactulose, mannitol and sucrose concentration determination were drawn on day 0, 2, 6, 11 and 20. Lactulose/mannitol (L/M) index was calculated. Treatment with meloxicam and meloxicam with misoprostol resulted in lower PGE_2 and PGI_2 serum concentrations in comparison to the placebo. L/M index and sucrose plasma concentration were increased in both groups in comparison to the placebo. According to the results of the study, meloxicam has altered gastrointestinal permeability and depleted prostaglandins production. Misoprostol was shown to be an effective preventing treatment.

Key words: dogs, gastrointestinal permeability, L/M index, NSAID, prostaglandins, sucrose

INTRODUCTION

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) in small animal medicine includes the treatment of osteoarthritis, acute traumatic injuries of the musculoskeletal system, acute inflammation, arteriolar thromboembolism, pain management in case of meningitis, bone tumours and soft tissue swelling or

injury. They are also used as perioperative analgesics for pain associated with surgical procedures and a variety of other disorders that require short- or long-term mild analgesia (Mathews, 1996; Jones and Budsberg, 2000; Lees *et al*., 2004; Lascelles *et al*., 2005). The use of NSAIDs in dogs is frequently limited due to gastric irritation and ulceration after oral administration (Bowersox *et al*., 1996; Forsyth *et al*., 1998; Buttgereit *et al*., 2001). The damage can range from mild erosive disease of uncertain clinical importance to complicated ulcer disease with attendant risks of bleeding and perforation. These problems are usually very difficult to manage in animals, because mild clinical signs of disease are easily overlooked (Meddings *et al*., 1995; Forsyth *et al*., 1998). The mechanism of gastric injury is complex and involves many factors. NSAIDs cause the damage of the gastrointestinal mucosa by direct topical injury, which is the first step in NSAID ulceration and by systemic effects mediated by depletion of cyclooxygenase-1 (COX-1) derived endogenous prostaglandins (Thjodleifsson and Bjarnason, 1999; Schneider *et al*., 1999; Rich and Scheiman, 2000; Lichtenberger, 2001; Tomlinson and Blikslager, 2003). COX-1, the constitutive isoform of the COX (cyclooxygenase) enzyme (the rate limiting enzyme for synthesis of eicosanoids such as prostaglandins (PGs), prostacyclins and thromboxanes from arachidonic acid), is believed to be responsible for the basal physiologic functions provided by the PGs (Mathews, 1996; Jones and Budsberg, 2000; Wallace and Li Ma, 2001; Brideau *et al*., 2001; Tomlinson and Blikslager, 2003; Lascelles *et al*., 2005). Conversely, cyclooxygenase-2 (COX-2), the inducible isoform of the COX enzyme, is thought to be responsible for the inflammatory activity of prostaglandins (Steinmeyer, 2000; Jones and Budsberg, 2000; Tomlinson and Blikslager, 2003; Lescelles *et al*., 2005; Sessions *et al*., 2005). It can be summarized that the therapeutic anti-inflammatory, analgesic, and antipyretic effects are due to the inhibition of COX-2. The undesirable gastrointestinal adverse effects, nephrotoxicity and coagulation disorders are believed to result from inhibition of COX-1 (Vane and Botting, 1998; Steinmeyer, 2000; Lichtenberger, 2001; Jones *et al*., 2002; Boston *et al*., 2003). Inhibition of COX-1 with a resultant decrease in endogenous prostaglandins critical to mucosal defence, especially PGE_1 (prostaglandin E_1), PGE_2 (prostaglandin E_2) and PGI_2 (prostaglandin I_2), is thought to be the most important mechanism of action of NSAIDs (Bowersox *et al*., 1996; Rich and Scheiman, 2000; Tomlinson and Blikslager, 2003). These together induce a subsequent increase in the permeability of the mucosa to toxins and luminal agents such as bile, pancreatic secretion, and bacteria (Bjarnason and Peters, 1996; Reuter *et al*., 1997; Thjodleifsson and Bjarnason, 1999). Gastroduodenoscopy has been the gold standard assay for NSAID-induced epithelial damage in human medicine. However, it is unsuitable as a routine screening test for diagnosis of upper gastrointestinal damage induced by NSAIDs in dogs as it is limited to the stomach and duodenum and requires anaesthesia. It is invasive, time-consuming, and expensive and may not be available in all clinics (Meddings *et al*., 1993; Sutherland *et al*., 1994; Davies, 1998; Davies and Saleh, 2000; Craven *et al*., 2007).

Gastrointestinal permeability tests have been demonstrated to be useful in both basic and clinical studies for the investigation of gastrointestinal damage

induced by NSAIDs (Davies, 1998; Craven *et al*., 2007). Increased gastrointestinal permeability is a hallmark of several disease processes that culminate in epithelial damage (Meddings *et al*., 1995). The administration of site specific permeability probes, such as monosaccharides and disaccharides, to detect permeability defects at different levels of the gastrointestinal tract represent a single screening test for assessment of the functional integrity of the gastrointestinal mucosa (Meddings and Gibbons, 1998). One or more sugar probes are given orally and the excretion of the probe(s) is than measured in the urine, plasma or serum (Meddings *et al*., 1993; Bijlsma *et al*., 1995; Van Elburg *et al*., 1995; Fleming *et al*., 1996; Cox *et al*., 1997; Sorensen *et al*., 1997; Johnston *et al*., 2000; Uil *et al*., 2000; Smecuol *et al*., 2001; Craven *et al*., 2007).

Meloxicam is considered to be a COX-2 preferential (Smecuol *et al*., 2001; Plumb, 2002; Jones *et al*., 2002; Boston *et al*., 2003), and spare COX-1 activity as confirmed by *in vitro* and *in vivo* studies (Engelhardt *et al*., 1996a; Engelhardt *et al*., 1996b; Kay-Mugford *et al*., 2000; Brideau *et al*., 2001; Jones *et al*., 2002). The relative selectivity of meloxicam for COX-2 may contribute to an improved tolerability profile compared with less selective NSAIDs (Vane, 1995; Fresno *et al*., 2005), resulting in reduced mucosal injury after its use (Smecuol *et al*., 2001). Misoprostol is a synthetic prostaglandin E_1 analogue that inhibits gastric acid production and has a cytoprotective effect (Bauer, 1985; Walt, 1992; Johnston *et al*., 1995; Bowersox *et al*., 1996). It has been shown to prevent the development of gastric mucosal lesions in humans and dogs during NSAID administration (Walt, 1992; Johnston *et al*., 1995; Bowersox *et al*., 1996; Ward *et al*., 2003).

On the contrary to human medicine, there is still a lack of studies and experimental data about the effective use of misoprostol as a gastro protective agent in NSAIDs induced gastrointestinal complications in dogs. Therefore, the present study aims to determine the effect of short-term use of meloxicam on gastrointestinal mucosa and the effectiveness of misoprostol in preventing meloxicam induced gastrointestinal damage in healthy dogs by means of PGE_2 and PGI_2 concentration, lactulose/mannitol (L/M) index and sucrose concentration determination.

MATERIAL AND METHODS

Animals

Seven adult beagle dogs with average body weight of 19.3 kg belonging to a research colony at the Veterinary Faculty, University of Ljubljana, Slovenia, were included in this study. All dogs entered the study upon normal findings on a physical examination and laboratory results of complete cell count, white cell differential determination and serum biochemical analysis within the reference range (data not shown). Prior to entering the study all dogs were treated for potential gastrointestinal parasites with praziquantel and febendazole (Zantel®, CPML, Galway, Ireland). The study protocol was reviewed and approved by the Ministry of Agriculture, Forestry and Food, Veterinary Administration of the Republic of Slovenia; licence No 323-02-221/2004/3.

Study protocol

The study was divided into three different treatments, each lasted for 20 days. All 7 dogs participated in each treatment. One day before the first treatment basal values for serum PGE_2, PGI_2, plasma sucrose concentration and L/M index were determined for all seven dogs. On first treatment the dogs (placebo group) were treated for ten days with placebo (Aqua pro injectione Braun®, B Braun, Melsungen, Germany), 3 mL PO q24h, on the second treatment the dogs (meloxicam group) were treated for ten days with meloxicam (Metacam®, Vetmedica, Boehringer Ingelheim, Germany), 0.2 mg/kg of body weight, PO, q24h. On the third treatment the dogs (meloxicam-misoprostol group) were treated for ten days with meloxicam, 0.2 mg/kg of body weight, PO, q24h, together with misoprostol (Cytotec®, Searle, Pfizer, NY, USA), 0.1 mg/kg of body weight, PO, q8h. For a determination of the plasma sucrose level and the L/M index the dogs were fed a sugar solution containing 2 g of mannitol, 40 g of sucrose, 10 g of lactulose and water added up to 100 mL. The osmolarity of the sugar solution was 1560 mmol/L. The dogs were fed 100 mL of the sugar solution, preheated to room temperature, from a clean bowl after an over-night fast, 120 minutes after the treatment (according to the group).

From day 11 to day 20 the dogs did not receive any medication for all three treatment groups. There was a minimum of 14 days of resting period between the three consecutive treatments. Dogs were housed in individual cages, provided water *ad libitum*, and fed a commercially available diet (Pedigree Pal, Mars Incorporated, USA) twice a day. All dogs were monitored daily for evidence of vomiting, diarrhoea, depression, inappetence, or abdominal pain through the course of the study.

Blood sample collection and preparation

Blood samples for serum PGE_2 and PGI_2 measurement and determination of plasma sucrose, lactulose and mannitol concentration were collected from the jugular vein at each treatment on day 2, 6, 11 and 20 and one day before the first treatment for basal PGE_2, PGI_2, sucrose, lactulose and mannitol concentration determination. L/M index was calculated as a ratio between the concentration of lactulose and mannitol.

For a determination of the plasma sucrose level and the L/M index the blood samples were drawn from the jugular vein 120 minutes after ingestion of the sugar solution into two separate 2 mL sodium fluoride and K_3EDTA tubes (Vacuette, Greiner bio-one, Kremsmuenster, Austria). The tubes were than centrifuged at 1500 g for 15 minutes at 4°C. Plasma was harvested and stored at -80°C prior to analysis according to previously reported methods (Vovk *et al*., 2003; Pukl and Prošek, 1990). For the serum PGE_2 and PGI_2 measurement blood samples were collected from the jugular vein 120 minutes after treatment, before the sugar solutions were fed. The samples were collected into two separate 4 mL serum tubes (Vacuette, Greiner bio-one, Kremsmuenster, Austria). The tubes were left at room temperature, allowed to clot for 10 min and than centrifuged at 1300 g for 10 minutes at room temperature. Serum was harvested and stored at -20°C until analysis. Serum PGE_2 and 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2)

concentrations were determined by the use of monoclonal EIA kit (R&D Systems Inc., USA) according to the manufacturer's instructions.

Statistical analysis

Data analysis was performed using the SPSS computer program (SPSS 15.0 for Windows, Chicago, Illinois, USA). Results are expressed as mean ± standard deviation (mean ± SD). For each variable the data were examined for normality. Paired t-test with Bonferroni correction for multiple tests was used for comparison of measured parameters between placebo and meloxicam, placebo and meloxicam-misoprostol and between meloxicam and meloxicam-misoprostol group at different sampling times. Paired t-test was also used to compare basal values of all parameters with day 2 values of placebo, meloxicam and meloxicam-misoprostol group. The minimum level of significance was defined at $p<0.05$.

RESULTS

In the meloxicam group, serum PGE_2 concentrations (Figure 1) were lower at a statistically significant level at days 2 and 6 of the treatment and at day 11, compared to the placebo group. In the meloxicam-misoprostol group, a statistically significant lower serum PGE_2 concentration was determined at day 11, when serum PGE_2 reached the lowest value of 3.50 ± 0.86 μg/L, in comparison with the placebo group's serum PGE_2 concentration of 7.49 ± 1.54 μg/L. However, no statistically significant differences in PGE_2 concentration between meloxicam and meloxicam-misoprostol group were found. A statistically significant difference between the basal values of serum PGE_2 and the meloxicam day 2 values of PGE_2 was found. At day 20 the serum PGE_2 concentration in meloxicam (5.81 ± 2.75 μg/L) and meloxicam-misoprostol group (5.16 ± 0.75 μg/L) increased near the placebo value (5.97 ± 1.01 μg/L), which is shown in Figure 1.

Similar to the serum PGE_2 concentrations, serum PGI_2 concentrations (Figure 2) were lower in both treatment groups from day 2 to day 11 in comparison to the placebo group. In the meloxicam group a statistically significant lower serum PGI_2 concentration was determined at day 6, while in the meloxicam-misoprostol group it was lower at days 2, 6 and 11. There were no statistically significant differences in the serum PGI_2 concentrations between the meloxicam and meloxicam-misoprostol groups. A statistically significant difference between the basal values of serum PGI_2 and the meloxicam-misoprostol day 2 values of PGI_2 was found.

Similar to the serum PGE_2 concentrations, serum PGI_2 concentrations increased at day 20 in both treatment groups with serum PGI_2 concentrations in meloxicam (8.56 ± 1.93 μg/L) and meloxicam-misoprostol group (8.54 ± 2.47 μg/L) above the placebo value (7.79 ± 1.20 μg/L).

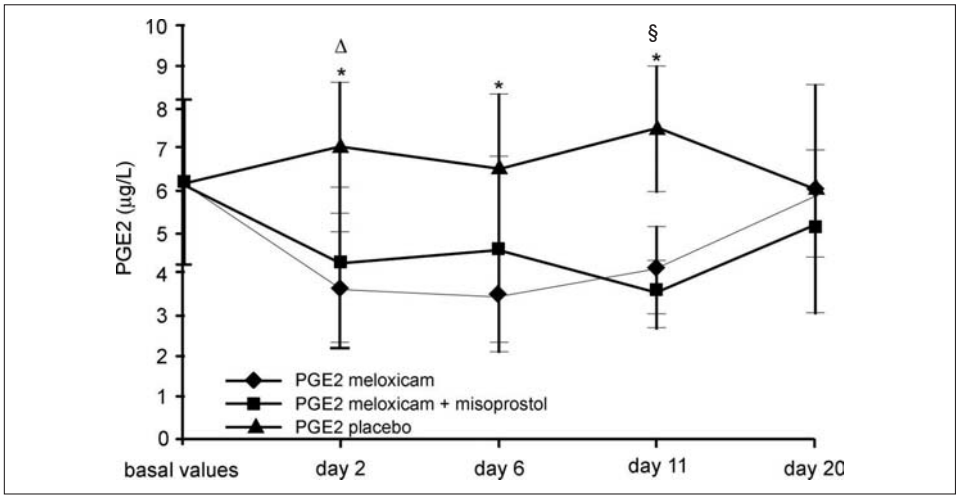

Figure 1. Serum PGE_2 concentrations (mean±SD) in the placebo, meloxicam and meloxicam-misoprostol group
* p<0.05 meloxicam group in comparison to the placebo
§ p<0.05 meloxicam-misoprostol group in comparison to the placebo
Δ p<0.05 basal values in comparison to the day 2 values of meloxicam group

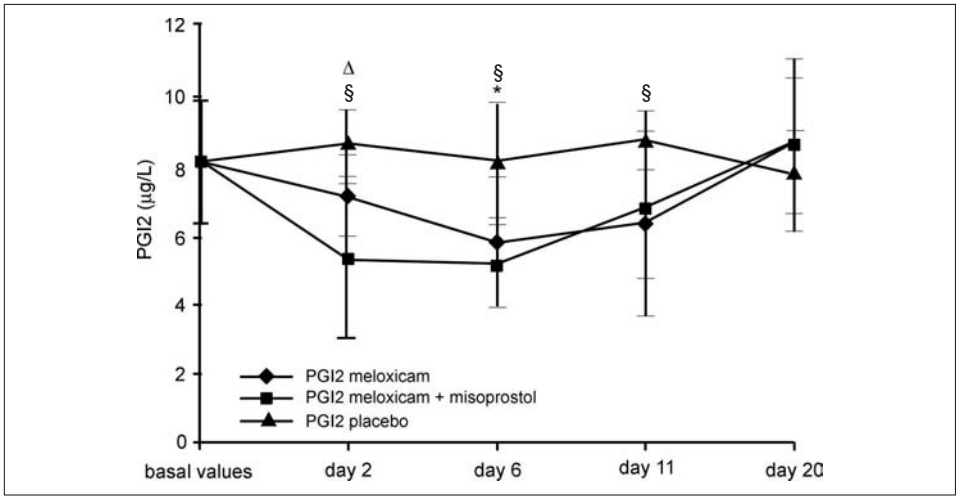

Figure 2. Serum PGI_2 concentrations (mean ± SD) in the placebo, meloxicam and meloxicam-misoprostol group
* p<0.05 meloxicam group in comparison to the placebo
§ p<0.05 meloxicam-misoprostol group in comparison to the placebo
Δ p<0.05 basal values in comparison to the day 2 values of meloxicam-misoprostol group

An increase of the mean value of the L/M index (Figure 3) with a high standard deviation was observed in the meloxicam group with the highest value at day 11 (L/M index of 0.038 ± 0.033), compared to day 2 L/M index in the placebo group with the value of 0.014 ± 0.007. The index returned back to the placebo values at day 20. There were no statistically significant differences in the meloxicam group in comparison to the placebo group. L/M index was higher in the meloxicam-misoprostol group (0.022 ± 0.009) at day 2 in comparison to the placebo (0.012 ± 0.001) and to the meloxicam group (0.016 ± 0.007). However, it reached the placebo level, 0.012 ± 0.006 versus 0.012 ± 0.003; as early as day 6 and remained at this level until day 20. There were no statistically significant differences in L/M index between the meloxicam and the meloxicam-misoprostol group. A statistically significant difference between the basal values of L/M index and the meloxicam-misoprostol day 2 values of L/M index was found.

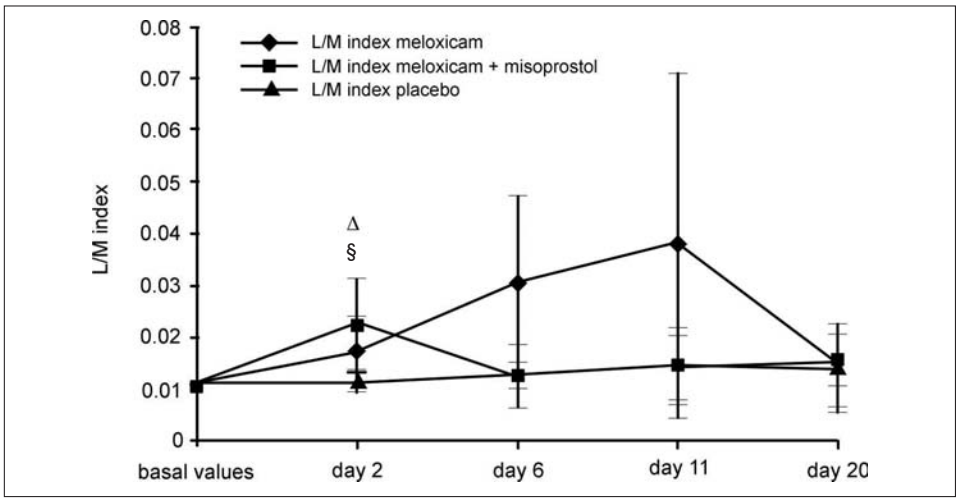

Figure 3. L/M index (mean ± SD) in the placebo, meloxicam and meloxicam-misoprostol group
§ $p<0.05$ meloxicam-misoprostol group in comparison to the placebo
Δ $p<0.05$ basal values in comparison to the day 2 values of meloxicam-misoprostol group

The concentration of sucrose (Figure 4) was statistically significantly higher on day 2 in the meloxicam, 21.87 ± 11.66 μg/mL and the meloxicam-misoprostol group, 28.7 ± 9.92 μg/mL, in comparison to 8.5 ± 2.55 μg/mL found in the placebo group. On day 6, sucrose in the meloxicam group reached a peak concentration of 31.90 ± 18.57 μg/mL and decreased close to the placebo level around day 11 and remained so until day 20. In the meloxicam-misoprostol group sucrose concentration was the highest on day 2, 28.7 ± 9.92 μg/mL, but decreased near the placebo values, 14.00 ± 3.79 μg/mL versus 10.20 ± 1.79 μg/mL, at day 6 and remained at a similar concentration until day 20. There

were no statistically significant differences in sucrose concentration between the meloxicam and meloxicam-misoprostol group at any of the sampling times. A statistically significant difference between the basal sucrose concentration and the meloxicam-misoprostol day 2 sucrose concentration was found.

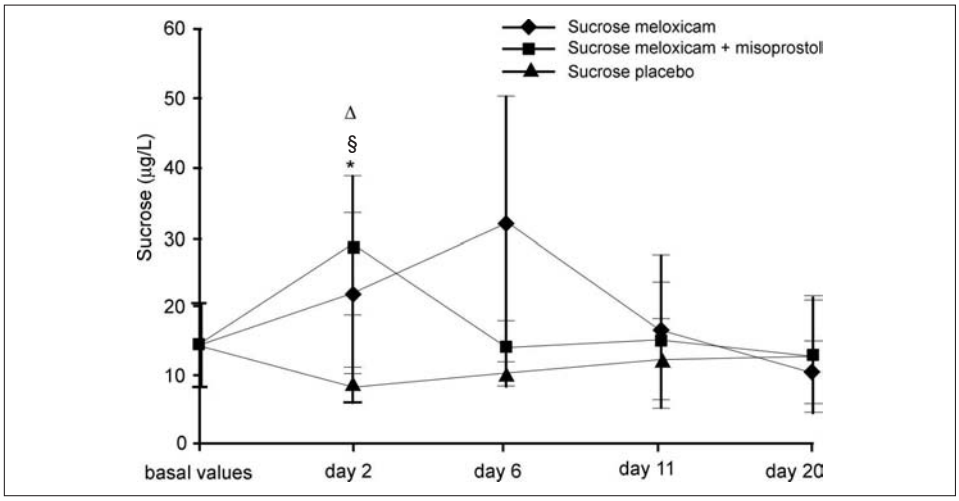

Figure 4. Sucrose concentration (mean±SD) in the placebo, meloxicam and meloxicam-misoprostol group
* $p<0.05$ meloxicam group in comparison to the placebo
§ $p<0.05$ meloxicam-misoprostol group in comparison to the placebo
Δ $p<0.05$ basal values in comparison to the day 2 values of meloxicam-misoprostol group

DISCUSSION

In the present study the effect of meloxicam on gastrointestinal mucosa and the effectiveness of misoprostol in preventing meloxicam induced gastrointestinal damage in healthy dogs were investigated. The main application of the study would be to support the benefit of concurrent administration of misoprostol with meloxicam in patients requiring frequent or short-term meloxicam treatment, or in patients at risk for gastrointestinal side effects associated with meloxicam use. With application of permeability tests in clinical practice it would be easier to investigate the possible gastrointestinal damage in canine patients after meloxicam use.

Meloxicam was chosen for this study as it is among the most commonly prescribed NSAIDs in veterinary medicine for treatment of acute and chronic pain associated with various conditions. Meloxicam is a NSAID, which has a more potent inhibitory activity against COX-2 than against COX-1. This makes meloxicam to be one of gastro intestinally safer NSAIDs (Poulsen and Horstermann, 1999; Plumb, 2002; Smecuol *et al*., 2001). However, its use is often

limited by its side effects such as gastric haemorrhage and ulceration, which leads to vomiting, abdominal discomfort or pain and diarrhoea. The later was also observed in our study during meloxicam and meloxicam-misoprostol treatment at different time points. Hypertonic sugar solutions that were used in the present study may also cause osmotic diarrhoea, distended abdomen and flatulence (Uil *et al*., 1997; Uil *et al*., 2000; Steiner *et al*., 2002), which were observed in four dogs soon after sugar solutions were ingested.

It is known that NSAIDs can affect gastrointestinal mucosa either by a local topical effect or via systemic actions. Systemically NSAIDs are thought to cause damage by inhibiting the cyclooxygenase system, thus preventing prostaglandin production via the arachidonic acid pathway (Thjodleifsson and Bjarnason, 1999; Rich and Scheiman, 2000; Lichtenberger, 2001; Tomlinson and Blikslager, 2003). Prostaglandins of the E, F and I types are found in abundance in the gastric and duodenal mucosa. These prostanoids have important regulatory functions for gastric blood flow, inhibition of gastric acid secretion, stimulation of epithelial cell renewal and enhancement of secretion of gastric mucus with increased protein content, all of which are necessary to maintain a healthy gastrointestinal mucosa (Miller, 1988; Vane, 1995).

In the present study serum prostaglandins were determined in order to prove possible systemic side effects of meloxicam on gastrointestinal mucosa and to investigate the cytoprotective action of misoprostol. A statistically significant decrease in serum PGE_2 and PGI_2 concentration in comparison to the placebo values were detected during the ten days of treatment with meloxicam which could be ascribed to the systemic effects of meloxicam on prostaglandin synthesis. Ten days after the treatment PGE_2 and PGI_2 values returned close to the placebo values. We can conclude that ten days after the treatment with meloxicam has been abolished there were no systemic effects on prostaglandin synthesis. Similar results were obtained in meloxicam-misoprostol group, where a statistically significant decrease in serum PGI_2 was observed during the ten days of treatment, as well as one day after the treatment. On the other hand a decrease near the statistical significance in serum PGE_2 was detected at day 2 and 6, and statistically significant decrease one day after the treatment, in comparison to the placebo group. However, we found no statistically significant differences between the meloxicam and meloxicam-misoprostol groups, when comparing the PGE_2 and PGI_2 serum concentrations. The results of the present study are in agreement with the results of similar studies, where the productions of prostaglandins after COX-2 selective NSAIDs administration were studied (Brideau *et al*., 1996; Tanaka *et al*., 2002; Sessions *et al*., 2005; Brainard *et al*., 2007). They support the concept that inhibition of so called "cytoprotective" prostaglandins synthesis by NSAIDs is a major factor in the development of gastric damage (Rich and Scheiman, 2000; Brideau *et al*., 2001; Lichtenberger, 2001; Wallace and Ma, 2001; Lascelles *et al*., 2005).

Misoprostol is a synthetic prostaglandin related structurally to naturally occurring prostaglandin E_1. Misoprostol has been shown to effectively decrease histamine, pentagastrin, and meal-stimulated gastric acid secretion in the dog. Proposed mechanisms by which mucosal protection from misoprostol may occur

include prevention of gastric mucosal barrier disruption, simulation of mucous and alkaline secretions, and enhancement of gastric blood flow (Bauer, 1985; Ward *et al*., 2003). Misoprostol has been shown to be effective in preventing gastric haemorrhage and in treating gastric ulcerations in humans receiving NSAIDs (Walt, 1992; Johnston *et al*., 1995; Steinmeyer, 2000; Buttgereit *et al*., 2001). It may also be effective in preventing gastric haemorrhage and ulcerations in dogs receiving NSAIDs (Murtaugh *et al*., 1993; Bowersox *et al*., 1996; Mathews, 1996; Ward *et al*., 2003).

Most commonly reported adverse effect for meloxicam and misoprostol use in dogs is gastrointestinal distress with vomiting and diarrhoea, but it apparently occurs only occasionally. It is usually transient and resolves within a few days (Poulsen and Horstermann, 1999; Plumb, 2002). Although some dogs in our study experienced mild transient gastrointestinal distress in both treatment groups it can be noted that those were adverse effects of drugs, since they resolved spontaneously and did not progress. Undesirable adverse effects of meloxicam may appear with minimal prodromal signs and symptoms that are often overlooked by the owner (Meddings *et al*., 1995; Forsyth *et al*., 1998). Therefore, there is a need for a simple screening test for animals, such as a sugar permeability test, to predict which animals are at risk for developing gastric damage, due to the fact that the ability to perform endoscopic surveillance in animals is often limited and gives subjective results (Sorensen *et al*., 1997; Davies, 1998; Meddings and Gibbon, 1998; Davies and Saleh, 2000). Simultaneous use of sucrose and lactulose/mannitol probes allows non-invasive detection of gastric and enteric damage, respectively (Meddings and Gibbons, 1998). The plasma (or serum) ratio between orally administered disaccharide lactulose and monosaccharide mannitol expressed as the lactulose/mannitol (L/M) index may be used as the indicator of intestinal permeability (Fleming *et al*., 1996; Van der Hulst *et al*., 1998; Bruet *et al*., 2008). Intestinal permeability increases with increasing L/M index. Sucrose and lactulose are both disaccharides of similar molecular size, and the permeation pathways of both are expected to be similar. The major difference is that sucrose, unlike lactulose is rapidly degraded within the small intestine by sucrase-isomaltase to its monosaccharide constituents, glucose and fructose. This makes sucrose a site specific permeability probe for gastro duodenal damage (Meddings *et al*., 1993; Sutherland *et al*., 1994; Smecuol *et al*., 2001).

Sucrose and lactulose/mannitol probes were used simultaneously in the study in order to detect gastric and enteric damage caused by NSAID meloxicam. Measurement of gastric permeability is greatly simplified by the realization that sucrose is a unique probe molecule for the determination of gastro duodenal permeability because the digestive process distal to the stomach effectively destroys it. Sucrose is insensitive to even severe small intestinal damage, provided that this is beyond the duodenum (Meddings *et al*., 1993). Meddings at al. (1995) reported that increased sucrose permeability is a reflection of generalized mucosal damage and not of endoscopically observed ulceration, due to the fact that epithelial damage viewed from the perspective of a molecule the size of a disaccharide and that evaluated by the endoscope are different.

Endoscopically observed ulcers are rarely found without widespread abnormalities in the gastric epithelium.

In the study presented a significant increase in sucrose permeability was observed at day 2 of the meloxicam treatment, though plasma sucrose reached the highest mean value around day 6. Steady-state conditions of meloxicam in the plasma would be found around this time (Busch *et al*., 1998). However, sucrose permeability dropped to almost placebo values on day 11 and even to lower values on day 20. Therefore, these results demonstrate that meloxicam alters permeability of gastric mucosa during the ten days of treatment, but its effect diminishes after the treatment has been abolished.

In the meloxicam-misoprostol group a significant increase in sucrose concentration was observed at day 2 of the treatment in comparison to the placebo group, consistent with the meloxicam group. However, sucrose concentration decreased to the placebo level as early as day 6 of the treatment (in comparison to the meloxicam group, where similar values were reached on day 11) and remained close to the placebo values until day 20, which confirms that misoprostol had a protective effect on the gastric mucosa. According to the literature, misoprostol was shown to be effective in preventing haemorrhage and ulcer development in dogs receiving NSAID (Murtraugh, 1993; Bowersox *et al*., 1996; Neiger, 2003; Ward *et al*., 2003), although comparison of the studies is not possible, since sucrose tests were not used in those studies.

The L/M index discriminates better between controls and patients with intestinal disease than the individual recoveries of these sugars. It has been shown to increase in humans with intestinal damage (Sorensen *et al*., 1997; Van der Hulst *et al*., 1998; Johnston *et al*., 2000). Intestinal epithelium is a heteroporous layer with a high incidence of small pores permitting the permeation of mannitol while excluding the passage of lactulose and a small population of larger pores – located in the tight junctions – allowing the permeation of both lactulose and mannitol (Uil *et al*., 2000). Advantages of the determination of a ratio include enhanced sensitivity because this evaluates not only the raised permeability to a larger disaccharide, due to the opening of intercellular pathways but also the effects of decreased absorption of a monosaccharide, due to reduced surface area. In addition the ratio helps to eliminate errors due to non-mucosal factors such as rate of gastric emptying and intestinal transit, as they would affect both sugars equally (Sorensen *et al*., 1997).

In the present study the L/M index in meloxicam-misoprostol group was higher on day 2 of the treatment in comparison with the placebo group; however it reached placebo values as early as day 6 and remained at the placebo level until day 20. On the contrary, the L/M index in the meloxicam group increased slightly from day 2 to day 11, when it reached the highest value. Data from the present study suggest that intestinal permeability is increased by NSAID meloxicam. L/M index was increased throughout the ten days of the treatment period with meloxicam and reached placebo values ten days after the treatment. Intestinal permeability was supposedly increased due to increased permeation of disaccharide lactulose which has resulted in a higher ratio between lactulose and mannitol. According to the results of the present study it can be concluded that

this effect can be reduced by concomitant administration of misoprostol, since L/M index in meloxicam-misoprostol group reached placebo values as early as day 6 of the treatment.

The results of the present study indicate that meloxicam alters gastro duodenal and small intestinal permeability and reduces serum PGE_2 and PGI_2 concentrations in dogs, therefore increasing the risk of developing gastrointestinal erosions, ulcerations and haemorrhages. According to the results of the study, we may conclude that PGE_1 synthetic analogue misoprostol is an appropriate preventative treatment for NSAID-induced gastrointestinal damage in dogs. It promotes specific protective mechanisms that are associated with the prevention of the gastrointestinal haemorrhage and erosions, which may accompany the administration of meloxicam in dogs.

Further studies that include endoscopy with histopathology of gastric and intestinal mucosa along with the sugar tests and prostaglandin measurement would give us a better insight whether the observed gastro duodenal and small intestinal damage after meloxicam use can represent a health risk for canine patients.

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UTICAJ MELOKSIKAMA I MELOKSIKAMA SA MIZOPROSTOLOM NA KONCENTRACIJU SERUMSKIH PROSTAGLANDINA I PROPUSTLJIVOST GASTROINTESTINALNOG TRAKTA KOD ZDRAVIH PASA RASE BIGL

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SADRŽAJ

Cilj ovog istraživanja je bio da se istraži efekat meloksikama i meloksikama sa mizoprostolom na koncentraciju prostaglandina E_2 (PGE_2) i prostaglandina I_2 (PGI_2) u serumu, kao i na propustljivost sluzokože gastrointestinalnog trakta. Nesteroidni antiinflamatorni lekovi, kao što je meloksikam, imaju neželjene efekte na gastrointestinalni trakt, koji su posledica deplecije prostaglandina i površinskih lezija. U ovo istraživanje je bilo uključeno sedam odraslih pasa rase bigl. Izvedena su tri različita tretmana koja su trajala po 20 dana (placebo, meloksikam i meloksikam i mizoprostol). Istih sedam pasa je učestvovalo u sva tri tretmana. Od 1. do 10. dana psi su primali placebo, meloksikam ili meloksikam zajedno sa mizoprostolom PO. Psi su zatim praćeni od 11. do 20. dana. Uzorci za određivanje koncentracije PGE_2 i PGI_2 u krvnom serumu, laktuloze, manitola i saharoze u krvnoj plazmi uzimani su 0, 2, 6, 11 i 20 dana ogleda. Takođe je izračunavan indeks laktuloza/manitol (L/M). Tretman meloksikamom sa mizoprostolom je imao za posledicu niže vrednosti koncentracije serumskih PGE_2 i PGI_2 u poređenju sa placebom. L/M indeks i koncentracija saharoze u krvnoj plazmi su bili povećani u obe grupe pasa u poređenju sa vrednostima registrovanim pri aplikaciji placeba. Naši rezultati ukazuju da meloksikam menja propustljivost u gastrointestinalnom traktu i smanjuje produkciju prostaglandina. Mizoprostol se pokazao kao efekasno preventivno sredstvo u ovom smislu.