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Short Communication

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Abstract

This randomized, double-blind, placebo-controlled study investigated the effects of glucosamine/chondroitin sulfate, chondroitin sulfate, and diacerein on the gastric mucosa of dogs, and on clinical signs of vomiting and diarrhea. Twenty-one healthy adult dogs were included in the study, and were randomly assigned to seven treatment groups, 3 dogs per group. The effect of those medicines on the gastric mucosa was evaluated by endoscopy on day 3 and day 14, and compared with pre-treatment. Clinical signs, including vomiting and diarrhea, were recorded every day during the study period. The results showed a non-significant effect of glucosamine/chondroitin sulfate, chondroitin sulfate, and diacerein on gastric mucosal lesions in healthy dogs. One dog vomited after receiving glucosamine/chondroitin sulfate, but only for the first 2 days. All dogs receiving diacerein showed symptoms of diarrhea during the 14-day trial. In conclusion, glucosamine/chondroitin sulfate, chondroitin sulfate, and diacerein at doses of 1,500 and 3,000 mg/day are gastric-safe for use in dogs.

Keywords: chondroitin sulfate, diacerein, dog, gastric mucosa, glucosamine

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บทคัดย่อ

การประเมินเยื่อบุผิวกระเพาะอาหารด้วยกล้องส่องเพื่อประเมินความปลอดภัยในการใช้ยาปกป้องกระดูกอ่อนผิวข้อ 3 ชนิด ในสุนัขสุขภาพดี

กรกฎ งานวงศ์พาณิชย์ [1,2] กิตติพงษ์ คุณประทุม [3] เทิดศักดิ์ ญาโน [4] กัมปนาท สุนทรวิภาต [5]*

ศึกษาแบบสุ่มตัวอย่างเปรียบเทียบผลของยากลูโคซามีนซัลเฟตผสมคอนดรอยติน ยาคอนดรอยตินซัลเฟต และยาไดอะซีรีน ต่อเยื่อบุกระเพาะอาหารและอาการทางคลินิก แบ่งสุนัขสุขภาพดีจำนวน 21 ตัว โดยการสุ่มเป็น 7 กลุ่ม (3 ตัวต่อกลุ่ม) ศึกษาผลของยาที่มีต่อเยื่อบุผิวกระเพาะอาหารด้วยกล้องส่องทางเดินอาหารในวันที่ 3 และ 14 หลังจากกินยา เปรียบเทียบกับก่อนกินยา และเก็บข้อมูลอาการทางคลินิกประกอบด้วยอาเจียนและท้องเสีย จากการศึกษาพบว่า ยาทั้ง 3 ชนิดไม่มีผลต่อเยื่อบุผิวกระเพาะอาหาร สุนัข 1 ตัวมีอาการอาเจียน 2 วันแรกหลังจากได้รับยากลูโคซามีนซัลเฟตผสมคอนดรอยติน สุนัขทุกตัวที่ได้รับยาไดอะซีรีนมีอาการถ่ายเหลวตลอดระยะเวลาที่ทำการศึกษา ผลจากการศึกษาแสดงให้เห็นว่าการให้ยาทั้ง 3 ชนิดในขนาด 1,500 และ 3,000 มก. ต่อตัวไม่ผลต่อเยื่อบุกระเพาะอาหาร

คำสำคัญ: คอนดรอยตินซัลเฟต ไดอะซีรีน สุนัข เยื่อบุกระเพาะอาหาร กลูโคซามีน

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Introduction

Medications for treatment of osteoarthritis (OA) can be classified into two groups: symptom-modifying and disease-modifying drugs (McNamara et al., 1997). Symptom-modifying drugs include non-steroidal anti-inflammatory drugs (NSAIDs), while disease-modifying or chondroprotective drugs include glucosamine, chondroitin sulfate, diacerein and tetracycline. These chondroprotective drugs are commonly prescribed for treatment of OA because they are able to control metabolism in OA-joint by decreasing catabolism and increasing anabolism. A number of research studies have presented the effects of these drugs on improving the pathology of OA in humans and in animals as well (Olsen, 2011; Davies et al., 2013). However, there are many questions that arise when using these drug, for example, which is the most effective, over how long a period of time should they be used, how often should they be taken, and at what concentration. Although some of these questions have been partially answered, no drug has been clearly demonstrated to be more effective than the others; the drug must also be used for a lifetime, at a concentration depending on clinical signs. Thus far, clinical studies have found little evidence of adverse side effects from the use of chondroprotective drugs (Leffler et al., 1999; Brandt et al., 2005; Nganvongpanit et al., 2009).

In a human study that graded the efficacy of chondroprotective drugs, glucosamine, chondroitin sulfate and diacerein were classified as 'platinum', which indicated that there were good evidence for their effectiveness in the treatment of OA (Bruyère et al., 2008). Glucosamine is an aminosaccharide, acting as a preferred substrate for the biosynthesis of glycosaminoglycan chains and, subsequently, for the production of aggrecan and other proteoglycans of cartilage. It can increase matrix structural protein turnover, with catabolism being predominant over synthesis (Reginster et al., 2005).Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) and acts as a major component of proteoglycans of the extracellular matrix of connective tissues. The most effective property of chondroitin sulfate in OA-joint is pro-anabolic and anti-catabolic effect on, although chondroitin sulfate also increases cell viability and demonstrates anti-inflammatory properties (Hochberg et al., 2013). Diacerein (9, 10-dihydro-4, 5-bis (acetyloxy)-9, 10-dihydro-9, 10-dioxo-2-anthracene carboxylic acid) acts as an IL-1β blocker, inhibiting the IL-1β-stimulated MMP-3 and collagenase activity (Martel-Pelletier and Pelletier, 2010).

Even though, no certain dose of chondroprotective drugs is reported, most recommend approximate doses between 500-1,500 mg per dog per day. Moreover, gastric irritation is one of the concerns when using these drugs especially when using high drug concentration to treat moderate to severe OA (Davies et al., 2013). However, no research

has focused on the effect of these drugs on gastric mucosa when used in high dose; previous studies reported only clinical signs after receiving drugs. therefore, this study aimed to investigate the effect of two difference doses (1,500 and 3,000 mg per dog) of three chondroprotective drugs including glucosamine, chondroitin sulfate and diacerein on the gastric mucosa by clinical signs and endoscopic evaluation.

Materials and Methods

The study protocol was approved by the ethics committee of the Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand, in 2012. Twenty-one healthy adult dogs (male = 10, female = 11) volunteered for the study, based on a normal physical examination, unremarkable serum chemistry (liver and kidney) and complete blood count (Table 1). All dogs participating in the study had no history of gastrointestinal disorders, for example, vomiting and diarrhea for at least 2 months prior to the study. The dogs did not receive any medicine for 1 month before entering the study. The pool of 21 dogs were randomly double-blind assigned to seven treatment groups (Table 2). Three chondroprotective drugs, glucosamine/chondroitin sulfate (Synoquin®; Vet plus, England), chondroitin sulfate (Fortiflex®; Virbac, France), and diacerein (Artrodar®; TRB Chemedica, Switzerland), were used in the study, while gelatin capsules served as a placebo (control). Medicines were administered once a day, after evening meal. All dogs in the study were fed only dry food twice a day, morning and evening. Additional food and medicine were restricted during the study period.

The effect of these chondroprotective drugs on the gastric mucosa was evaluated using endoscopy (Schölly Fiberoptic, Germany) on day 3 and day 14, and the results were compared with pre-treatment. Moreover, clinical signs, including vomiting and diarrhea, were recorded every day during the study period. Gastroscopy was performed under general anesthesia. All animals first received 0.04 mg/kg atropine sulfate (TP Drug Lab, Thailand) by intramuscular injection as a preanesthetic agent. Propofol® (B. Braun, Germany) was administered at a 3 mg/kg intravenous dosage as a general anesthesia inducer. The animals were left under spontaneous and mechanical ventilation with oxygen at 100 vol%, maintained with 1% to 3% isoflurane inhalation anesthetic (Terrell™; Minrad, USA). During evaluation of the stomach, veterinarians were blinded to the group classification of dogs.

Gastric mucosal lesions were scored on the basis of a 12-point scale, as described in Baan et al, (2011) (Table 3). The stomach was divided endoscopically into four anatomical regions: 1) pylorus and pyloric antrum; 2) incisura angularis, extending along the lesser curvature; 3) greater curvature from the cardia to the pyloric antrum; and 4) cardia, extending from the greater curvature region to the lesser curvature that was not included with the incisura angularis (Moreau et al., 2005). R statistical software was used to analyze the study results. Fisher test function was applied for analysis of the relationship between different treatments and endoscopic scores, as well as instances of vomiting and diarrhea.

Table 1 Range of complete blood counts and blood chemistry pre-treatment

	Standard value*	Dogs
Hematocrit (%)	37-55	45±4
Hemoglobin (g/dl)	12-18	15±2
RBC (x10^6 cells/mm^3)	5.5-8.5	6.2±1.2
WBC count (cell/µl)	6,000-17,000	8,855±3,512
Neutrophil (%)	60-77	68±5
Lymphocyte (%)	12-30	20±4
Monocyte (%)	3-10	3±2
Eosinophil (%)	2-10	1±2
Basophil (%)	0-1	1±1
Alkaline Phosphatase (mg/dl)	20-120	44±27
ALT (mg/dl)	5-50	32±15
BUN (mg/dl)	10-22	27±14
Creatinine (mg/dl)	0.4-1.5	0.7±0.4

* Standard value from Veterinary Central Laboratory, Veterinary Diagnostic Laboratory, Chiang Mai, Thailand

Table 2 Experimental groups in the study

Group	**Treatment**	**N (male/female)**	**Weight (kg)**	**Age (months)**
GC-1500	Glucosamine/ chondroitin sulfate 1,500 mg/day	3 (2/1)	19.30±3.20	44.33 ± 8.02
GC-3000	Glucosamine/ chondroitin sulfate 3,000 mg/day	3 (1/2)	21.33±4.04	45.33 ± 15.95
C-1500	Chondroitin sulfate 1,500 mg/day	3 (1/2)	20.33±3.21	40.33 ± 12.58
C-3000	Chondroitin sulfate 3,000 mg/day	3 (1/2)	20.67±2.08	45.00 ± 13.75
DAR-1500	Diacerein 1,500 mg/day	3 (2/1)	19.77±3.42	44.33 ± 16.26
DAR-3000	Diacerein 3,000 mg/day	3 (2/1)	20.77±2.80	41.33 ± 13.61
Control	Placebo	3 (1/2)	19.90±4.08	37.67 ± 16.07

Results and Discussion

Significant level was set at $p < 0.05$. During the study, vomiting was observed on days 1 and 2 in one member (1/3) of the GC-3000 group, which received 3,000 mg/day glucosamine/chondroitin sulfate ($p > 0.05$), but this clinical sign disappeared after day 3; none of the dogs in the other four groups showed this side effect. In a human study, vomiting was reported in 0.83% of patients receiving glucosamine/chondroitin sulfate (Kelly, 1998), while the side effects of the other two medicines were not reported.

Soft stools or diarrhea were found in the diacerein groups, which the dose of 1,500 or 3,000 mg/day (DAR-1500 and DAR-3000) were administered. All dogs in the DAR-1500 group had diarrhea from day 1 to day 9 ($p < 0.01$); on day 10, diarrhea was found in 2 dogs ($p < 0.01$), and on days 11-14 in 1 dog ($p > 0.01$). In the DAR-3000 group, 3 dogs showed symptoms of diarrhea during days 1–10 ($p < 0.01$), and 2 dogs during days 11-14 ($p > 0.01$). This side effect was not found in the other two groups. A previous study reported that 2.48% of human patients receiving glucosamine (Kelley, 1998) had diarrhea. The cause of diarrhea after receiving diacerein is not well understood; however it is believed that this may be due to the chemical structure of diacerein and rhein, which are anthraquinone derivatives (Nicolas et al., 1998). Anthraquinone is a laxative agent, and for this reason diacerein has a laxative effect as well. In humans, a high percentage of patients receiving diacerein initially showed symptoms of diarrhea. However, this side effect decreased over long-term use.

Total gastroscopy scores for all groups on days 3 and 14 were not significantly different ($p > 0.05$) compared to day 0. Only 1 dog receiving glucosamine/chondroitin sulfate had a gastric mucosal lesion score of 2 on day 3 of the experiment, and 1 dog receiving chondroitin sulfate 3,000 mg/day had a gastric mucosal lesion score of 1.3 on day 14 (Table 4).

Table 3 Endoscopy scoring scale

Grade	Scale	Grade	Scale
1	Normal, no visible lesions	7	3–5 erosions
2	1 mucosal hemorrhage	8	>5 erosions
3	2–5 mucosal hemorrhages	9	1 ulcer
4	>5 mucosal hemorrhages	10	2 ulcers
5	Diffuse mucosal hemorrhage	11	3 or more ulcers
6	1–2 erosions	12	Perforated ulcer

Erosion: superficial discontinuation of the mucosal epithelium.

Ulcer: lesion producing wide discontinuation of the mucosa with a central defect and a raised margin.

This randomized, double-blind, placebo-controlled study showed the non-significant effect of glucosamine/chondroitin sulfate, chondroitin sulfate and diacerein on the gastric mucosal lesions in healthy dogs. Moreover, a high dose (3,000 mg/day) of glucosamine/chondroitin sulfate could irritate the stomach and cause vomiting, but this occurred only for the first few days, after which this side effect disappeared. Another side effect found during the study was that the use of a high dose of diacerein could cause soft stools. However, the overall results of this study demonstrated the safety of glucosamine/chondroitin sulfate, chondroitin sulfate, and diacerein when administered in doses of 1,500 and 3,000 mg/kg, after which no gastric mucosal lesions were observed.

Table 4 Gastroscopy scoring scale

Group	Day 0	Day 3	Day 14
GC-1500	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
GC-3000	1.0 ± 0.0	1.3 ± 0.6	1.0 ± 0.0
C-1500	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
C-3000	1.0 ± 0.0	1.0 ± 0.0	1.3 ± 0.6
DAR-1500	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
DAR-3000	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Control	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
***P* value**	nd	1.00	1.00

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