

## Effect of Serum IL-1beta of PCSO-524 and Firocoxib in Dogs Undergoing Medial Patellar Luxation Repair

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### *Abstract*

Thirty client-owned Pomeranians, between one and seven years old that had grade-three medial patellar luxation, were enrolled in this experiment. The dogs did not have other diseases. All the dogs received the same physical and clinical orthopaedic examinations, as well as the peak vertical force gait analysis, radiographic examination for assessment of bone deformities. Then, blood was collected from each dog for evaluation of complete blood counts (CBCs), blood chemistry and biomarker assays (IL-1 $\beta$ ) before surgery. After surgery to correct their medial patellar luxations, they were divided into three groups by random sampling. The first group received only Firocoxib (5 mg/kg once daily). The second group received only PCSO-524 (10 mg/kg, twice daily). The third group received Firocoxib (5 mg/kg, once daily) combined with PCSO-524 (10 mg/kg, twice daily). Treatment finished at the end of the second week. Every dog received a blood collection before surgery and at days one, five and 14 after surgery. IL-1 $\beta$  concentrations in serum were determined by commercially available canine ELISA kits. The result was that two treatments (Firocoxib only and Firocoxib combined with PCSO-524) can reduce the serum IL-1 $\beta$  level by day 14 after the surgical correction of patellar luxation. However, the group that received PCSO-524 did not have significantly decreased of serum IL-1 $\beta$ , but it had a slower decline of the serum IL-1 $\beta$  level. We concluded that PCSO-524 did not diminish serum IL-1 $\beta$  levels as good as NSAID in case of post-surgical correction of patellar luxation. For the further study, we recommend to increase dose of PCSO-524 and period of treatment before surgery in other condition that causes acute inflammation. Maybe the extraction of active ingredients in PCSO-524 is necessary. Moreover, we suggest increasing the sample size for showing the more significance and accurate results.

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**Keywords:** acute inflammation, dog, Interleukin 1, patellar luxation, PCSO-524

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## Introduction

PCSO-524 is extracted from the New Zealand green-lipped mussel and is a popular nutraceutical agent for use in humans. It contains mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are classed as omega 3 polyunsaturated fatty acids (Sinclair et al., 2000). Many researches have shown that PCSO-524 can reduce pain and the progression of joint diseases such as rheumatoid arthritis and osteoarthritis in humans (Lau et al., 2004; Brien et al., 2008; Coulson et al., 2012). In dogs, PCSO-524 improves clinical signs of osteoarthritis and degenerative spinal diseases (Mongkon and Sontornvipart, 2012). The mechanism of this agent is inhibition of membrane arachidonic acid metabolism by blocking 5-lipoxygenase (LOX), which causes the formation of leukocyte B4 (LTB4) by polymorphonuclear cells (PMN) in vitro (Halpern, 2000). PCSO-524 also inhibits prostaglandin E2 (PGE2) production and thromboxane by inhibiting cyclooxygenase (COX) pathways (McPhee et al., 2007). Prostaglandin and thromboxane play the major role in the progression of cartilage degeneration.

Inflammation has an important role in pathogenesis and in clinical signs of joint diseases in both humans and animals. Many studies interpreted results from physical examinations, radiography, anthropometric measurements or assessments from patients (Lau et al., 2004; Brien et al., 2008; Coulson et al., 2012), while some studies measured the biomarkers (Murphy et al., 2006). Errors from physical examination, radiography, anthropometric measurement and assessment may be possible. The accuracy of data is an important factor in research, thus the measurement of biomarkers, the objective data, is more reliable. In human, IL-1 is a popular biomarker to detect joint inflammation. IL-1 plays an important role in bone loss in patients with rheumatoid arthritis (Dinarello, 2011). In addition, there are many studies showing that IL-1 is correlated with other diseases such as Alzheimer disease and cancer in human (Lewis et al., 2006; Shaftel et al., 2008). The study by Assuma and coworker in 1998 revealed that IL-1 antagonist can inhibit inflammatory response, by decreasing the recruitment of mononuclear cells, and bone loss in *Macaca fascicularis* with experimental periodontitis (Assuma et al., 1998). Another study found that IL-1 $\beta$  may be possible biomarker for detection of early stages of inflammation in dogs (Prachar et al., 2013).

PCSO-524 had been studying for controlling chronic inflammation in many diseases such as osteoarthritis or rheumatoid arthritis (Lau et al., 2004; Hurst et al., 2010; Coulson et al., 2012; Zawadzki et al., 2013). There are many researches supporting that PCSO-524 has good effect with this disease. However, there are just a few of the studies of PCSO-524 with acute inflammation. This is the first study of relation between using PCSO-524 and IL-1 $\beta$ , which is the inflammation-related biomarker in blood. The model of acute inflammation in this study is surgical correction of patellar luxation which we found mostly in orthopedic clinic. If PCSO-524 can reduce IL-1 $\beta$  as good as (or better than) NSAIDs, resulting in a reduction in the use of NSAIDs which generates more side-effects. Moreover, if PCSO-524 can inhibit acute inflammation, therefore a decrease in development from acute to chronic inflammation, can be possible.

The objective of this study was to compare the serum IL-1 $\beta$  levels in dogs undergoing patellar luxation surgery which is treated with PCSO-524, the extract from New Zealand Green-lipped mussel (*Perna canaliculus*) and/or Firocoxib.

## Materials and Methods

**Animal:** Thirty client-owned Pomeranians, between one and seven years old ( $3 \pm 2.4$  years) that had grade-three medial patellar luxation were enrolled in this experiment. The inclusion criteria were grade-three patellar luxation, no history of treatment for patellar luxation, no other diseases (eg. heart disease, skin disease) and normal blood results. Exclusion criteria were having other diseases, or an inability to receive drugs continuously.

Informed owner consent was gained and the research protocol was approved by the Faculty of Veterinary Sciences Ethics Committee, Chulalongkorn University, Bangkok, Thailand.

**Pretreatment evaluation:** All the dogs received the physical and clinical orthopaedic examinations and radiographic examinations (hip and stifle joints) to assess bone deformities. Three milliliters of blood was collected from each dog so that complete blood counts (CBCs), blood chemistry and biomarker assays (IL-1 $\beta$ ) could be performed.

**Table 1** Drugs using in anesthesia for surgery

	Drug	Concentration	Dose	Route
<b>Premedication</b>	Acepromazine	1 mg/ml	0.03 mg/kg	Intramuscular (combined)
	Morphine	10 mg/ml	0.5 mg/kg	
<b>Induction</b>	Propofol	10 mg/ml	4 mg/kg	Intravenous
<b>Maintainance</b>	Isoflurane	-	1-2 mg%	Inhalation
<b>Antibiotics</b>	Cefazolin	250 mg/ml	25 mg/kg	Intravenous
<b>Post-operative drug</b>	Tramadol	50 mg/ml	4 mg/kg	Subcutaneous

**Treatment procedure:** All dogs had an anesthesia protocol (Table 1) and received surgical treatment for the correction of patellar luxation by the same veterinary surgeon, that used three techniques (medial desmotomy, lateral imbrication and throclear block

recession) to correct medial patellar luxation. In the next day after surgery, they divided into 3 groups by random sampling. The first group received only Firocoxib (5 mg/kg once daily). The second group received only PCSO-524 (10 mg/kg, twice daily). The

third group received Firocoxib (5 mg/kg, once daily) combining with PCSO-524 (10 mg/kg, twice daily). Treatment finished at the end of the 2<sup>nd</sup> week.

**Parameter, monitoring and blood collection:** Every dog received a physical examination, an orthopaedic examination, a clinical scoring system, gait analysis and a pain-scoring system (Glasgow pain scale) by the same veterinarian before surgery and at days one, five and 14 after surgery. If the pain scale is more than 14, the dog was excluded from this research and relieved pain upon the veterinarian's decision immediately. Three milliliters of blood samples were collected from the cephalic vein or saphenous vein of each dog. One milliliter was separated into two tubes for complete blood counts (CBCs) and blood chemistry tests. The samples for CBCs were kept in anticoagulant 100IU/ml heparin (APSFInchem, Australia). The samples for blood chemistry were kept in plain tubes that did not contain anticoagulant. These samples were kept at 4°C. The other two milliliters of blood samples (for biomarker assays) were kept in plain tubes without anticoagulant and centrifuged at 7,000xg for 15 min to obtain serum, then stored at -20°C until a batch analysis was performed.

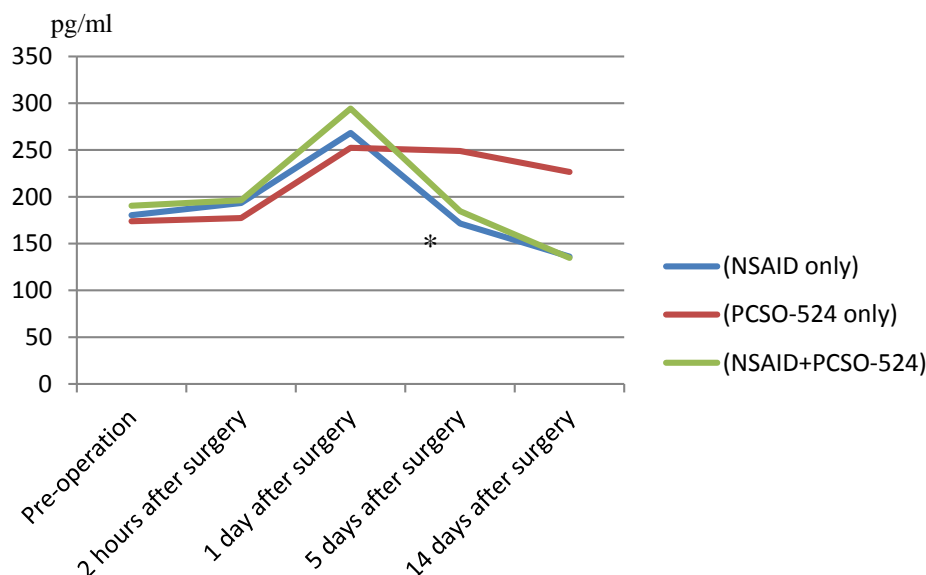
**Chemical substances' preparation and ELISA technique of IL-1 $\beta$ :** IL-1 $\beta$  concentration in serum will be determined by a commercially available canine ELISA kit. The IL-1  $\beta$  ELISA kit (USCN life Science Inc., China) has a minimum detectable dose that is typically less than 3.1pg/mL and a detection range of 7.81-500pg/mL. The microtiter plate has been

pre-coated with monoclonal antibody to canine IL-1 $\beta$ . Canine serum and Biotin-conjugated antibody specific to canine IL-1 $\beta$  were added to the wells consecutively. Therefore, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each well and incubated. After TMB substrate solution addition, only wells that contain IL-1 $\beta$ , Biotin-conjugated antibody and enzyme-conjugated Avidin showed a change in color. The enzyme-substrate reaction was terminated by addition of sulphuric acid solution. Finally, the color change was measured spectrophotometrically at a wavelength of 450 nm.

**Statistical analysis:** All data expressed as mean  $\pm$  SD. Parameters in group compared using repeated measure ANOVA (analysis of variance). Parameters between group compared by one-way ANOVA. P-values of <0.05 considered to be statistically significant. All data use SPSS programme to analyse.

## Results and Discussion

There was no significance of serum IL-1 level in 3 groups of treatment during 5 times of data collection. In all groups, serum IL-1 $\beta$  level increases in day 1 after surgery. In group 1 and 3, serum IL-1 $\beta$  level decreases significantly in day 14 after surgery comparing day 1 and 5 after surgery (\*), respectively. In group 2, serum IL-1 $\beta$  level slightly decreased since day 5 after surgery. However, there is no significantly difference of the value between day 1 and day 14 after surgery (Fig 1).



**Figure 1** Graph of serum IL-1 $\beta$  levels

This study show that two treatments (Firocoxib only and Firocoxib combined with PCSO-524) can reduce the serum IL-1 $\beta$  level by day 14 after the surgical correction of patellar luxation. However, the group that received PCSO-524 did not have significantly decreased of serum IL-1 $\beta$ , but it had a slower decline of the serum IL-1 $\beta$  level compared with the other treatments. This result did not relate with the

study by Singh et al. (2008), which reported that PCSO-524 can reduce acute inflammation. Their experiment indicated that the administration of crude lipid extract CO<sub>2</sub>-SFE and a free-fatty-acid extract from *Perna canaliculus* for five days was equipotent to the ability of Piroxicam to reduce inflammation in adjuvant-induced arthritis in rats (Singh et al., 2008).

In conclusion, PCSO-524 did not diminish serum IL-1 $\beta$  levels as good as NSAID in case of post-surgical correction of patellar luxation. For the further study, we recommend to increase dose of PCSO-524 and period of treatment before surgery in other condition that causes acute inflammation. Maybe the extraction of active ingredients in PCSO-524 is necessary. Moreover, we suggest increasing the sample size for showing the more significance and accurate results.

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## บทคัดย่อ

### ผลของสารสกัดจากหอยแมลงภู่นิวซีแลนด์และ Firocoxib ต่ออินเตอร์ลิวคิน 1 เบต้า ในซีรัมของสุนัขที่ได้รับการผ่าตัดแก้ไขสะบ้าเคลื่อน

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สุนัขพันธุ์ปอมเมอเรเนียน อายุ 1-7 ปี ไม่จำกัดเพศ ไม่มีปัญหาสุขภาพอื่นๆ นอกจากมีภาวะลูกสะบ้าเคลื่อนระดับ 3 จำนวน 30 ตัว จะได้รับการตรวจร่างกายทั่วไป, ตรวจทางออร์โธปิดิกส์, ตรวจการเดิน, การลงน้ำหนัก และ ตรวจเลือดหาสารสื่ออักเสบในเลือด (IL-1 $\beta$ ) ก่อนเข้ารับการผ่าตัดแก้ไขลูกสะบ้าเคลื่อน หลังการผ่าตัด สุนัขทั้งหมดจะถูกสุ่มแบ่งออกเป็น 3 กลุ่ม กลุ่มละ 10 ตัว ได้รับการรักษาเป็นระยะเวลา 14 วันติดต่อกัน โดยกลุ่มแรกจะได้รับยา Firocoxib ปริมาณ 5 มก.ต่อกก. วันละ 1 ครั้ง กลุ่มที่สองจะได้รับสาร PCSO-524 ปริมาณ 1 แคปซูล วันละ 2 ครั้ง และกลุ่มสามจะได้รับยา Firocoxib ปริมาณ 5 มก.ต่อกก. วันละ 1 ครั้ง ร่วมกับสาร PCSO-524 ปริมาณ 1 แคปซูล วันละ 2 ครั้ง ทำการตรวจเลือดหาสารสื่ออักเสบในเลือด (IL-1 $\beta$ ) ในวันที่ 1, 5 และ 14 ภายหลังจากผ่าตัด ผลปรากฏว่ากลุ่มที่ได้รับยา Firocoxib และกลุ่มที่ได้รับยา Firocoxib ร่วมกับ PCSO-524 มีปริมาณ IL-1 $\beta$  ที่ลดลงในวันที่ 14 หลังการผ่าตัดอย่างมีนัยสำคัญทางสถิติ แต่กลุ่มที่ให้สาร PCSO-524 อย่างเดียวนั้น ถึงแม้ว่าจะสามารถลดปริมาณ IL-1 $\beta$  ได้แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ สรุปได้ว่า PCSO-524 ไม่สามารถลดปริมาณ IL-1 $\beta$  ในกระแสเลือดได้เทียบเท่ากับ Firocoxib ในสุนัขที่ได้รับการผ่าตัดแก้ไขสะบ้าเคลื่อน อย่างไรก็ตามการศึกษาผลของ PCSO-524 ต่อภาวะอักเสบแบบเฉียบพลันนั้นยังมีไม่มากนักในปัจจุบัน ทางผู้วิจัยจึงแนะนำให้เพิ่มปริมาณการใช้ PCSO-524 และควรมีการให้ยาก่อนการผ่าตัด รวมถึงการศึกษาผลของ PCSO-524 ในกรณีอื่นที่มีการอักเสบแบบเฉียบพลัน และการเพิ่มขนาดของกลุ่มตัวอย่างจึงเป็นที่น่าสนใจในการศึกษาต่อไปในอนาคต

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