Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 20 No. 4 Ser. No. 69 2019

IRANIAN JOURNAL OF VETERINARY RESEARCH

Short Paper

Substance P as a potential biomarker of pain assessment in dogs

Yoon, J. S.[1]; Park, J.[1**]; Song, R.[1] and Yu, D.[2*]**

[1]Department of Veterinary Internal Medicine, College of Veterinary Medicine, Chonbuk National University, Iksan 54596, Korea; [2]Department of Veterinary Internal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Korea

[*]***Correspondence:*** *D. Yu, Department of Veterinary Internal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Korea. E-mail: yudh@gnu.ac.kr*

[**]These authors contributed equally in this study

(Received 17 Oct 2018; revised version 21 May 2019; accepted 25 May 2019)

Abstract

Background: Substance P (SP) is a neuropeptide that functions as a neuromodulator. It is released mainly in the brain stem and in nerve endings. **Aims:** The present study aimed to provide fundamental data that may be applied to the assessment of pain in dogs by evaluating their serum SP concentrations. **Methods:** Two groups of dogs were designated as pain groups that included 10 dogs with medial patella luxation (MPL) and 10 dogs with fractures, respectively, and 20 healthy dogs were enrolled as the control group. **Results:** The SP concentrations in the serum of the pain groups reached 485.5 ± 250.1 pg/ml that was significantly higher than those of the control group, which reached 116.4 ± 38.5 pg/ml. In particular, serum SP concentrations in dogs with fractures (663.3 ± 225.3 pg/ml) were significantly higher compared to those of dogs with MPL (307.8 ± 105.3 pg/ml), indicating that serum SP levels increased when the dogs experienced greater pain. **Conclusion:** These findings propose the possibility that SP might be a useful biomarker for pain assessment in dogs. The present study may provide fundamental data that can aid in future pain management in dogs.

Key words: Dog, Neurotransmitter, Pain assessment, Substance P

Introduction

The term “pain” is defined as a sensory and emotional experience associated with actual or potential tissue damage accompanied by the stimulation of specific nerves that have “pain” receptors (IASP Subcommittee on Taxonomy, 1979). Pain is one of the most important vital signs that needs to be assessed as a first step via a physical examination when a veterinary patient visits the hospital due to various diseases. However, as communication with non-verbal animal patients is difficult, the accuracy and experience of veterinarians are needed to evaluate pain. Various pain scoring systems, such as the visual analog scale, simple descriptive scale, Melbourne pain scale, and Glasgow composite measure pain scale, have been used for measuring pain levels in veterinary practice. These pain scoring systems provide quantitative physiological measurements that are based on behavioral changes observed in patients and various physiological factors (Bufalari *et al*., 2007; Reid *et al*., 2018). Although these pain scoring systems are largely used to assess and treat pain in non-verbal patients, there is a continuing need to develop a method that involves the quantification of a biomarker of pain levels experienced by animal patients. The identification of a biomarker for pain assessment might provide additional information that can aid in assessing pain in animal patients.

In terms of physiology, various peptides are known to function as neuromodulators within nociceptive pathways in the nervous system (Wei *et al*., 2012). Substance P (SP), the first discovered neuropeptide, is known to be a major factor involved in neurogenic pain and inflammation (Schmidt *et al*., 2013). Substance P is released from the dorsal horn of the spinal cord and regulates the nociceptive neurons. It is associated with the transmission and modulation of pain, and thus, it is released in larger amounts as part of the bodily processes associated with physical pain and intense stress (Jessell, 1982). Given this background, increased SP level in proportion to pain intensity was reported in a human study (Jang *et al*., 2011; Lisowska *et al*., 2016). In the present study, in order to investigate whether SP could be a potential biomarker for pain levels in dogs as was previously reported in humans, we analyzed the serum SP concentrations of dogs assigned to two groups based on the pain associated with their orthopedic diseases and of healthy dogs. Following this, the serum SP concentrations were compared among the groups.

Materials and Methods

The present study was conducted on 20 dogs that visited the local animal hospitals in Seoul for medical

check-ups due to pain induced by clinical conditions in 2015. All protocols involved in this study complied with the ethical principles of medical research, including the Declaration of Helsinki. The dogs that were enrolled in this study were assigned to two groups, designated as pain groups,that included dogs with medial patella luxation (MPL) and dogs with bone fractures, respectively. Twenty healthy dogs without any abnormal clinical conditions were enrolled as the control group. All the dogs underwent physical examinations, lab tests, and radiological tests in the hospital. Pain was estimated by physical examination and Pre-emptive Pain Scoring System. Blood and serum samples were analyzed using an automatic blood cell counter (MEK-6450; Nihon Kohden, Tokyo, Japan) and chemistry analyzer (FUJI DRI-CHEM 7000i; Fujifilm Corp, Tokyo, Japan). At their initial presentations at the hospital, blood samples were collected from the dogs by direct jugular venipunctures. Following this, complete blood cell (CBC) counts were performed to assess the counts of red blood cells, leukocytes, and platelets. In addition, the blood samples were centrifuged at 1300 × g for 10 min, and the subsequent serum biochemistry analyses included the 18 items that are generally evaluated (e.g., serum protein, alanine transaminase (ALT), and aspartate transaminase (AST)) and electrolytes (e.g., sodium (Na), potassium (K), and chloride (Cl)). Moreover, to further analyze the serum samples for serum SP concentration levels, sera were immediately frozen at -80°C until assay. The serum SP concentrations of the dog serum samples were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) method with the substance P EIA kit (ENZO Life Sciences, Farmingdale, USA) according to the manufacturer's instructions. The samples were analyzed in duplicate in a single assay, and the absorbance was read at 405 nm using a microtiter plate reader (BioTek, Winooski, VT, USA). The SP concentrations were compared among the groups, and Student's t-tests were used to determine statistical significance using SPSS version 18 (IBM, Armonk, NY). P-values <0.05 were considered statistically significant.

Results

In total, 40 dogs were examined, 20 in the control group and 20 in the pain groups (dogs in the MPL group and dogs in the fracture group). The average ages, genders, and breeds of the enrolled dogs are summarized in Table 1. Among the dogs with MPL, six were affected by right MPL, three by left MPL, and one by bilateral MPL. Among the dogs with fractures, there were two dogs with a left femur fracture, one with a right femur fracture, one with a left metacarpal fracture, one with a right metacarpal fracture, one with left radius and ulnar fractures, two with right radius and ulnar fractures, one with multiple pelvic fractures, and one with a left olecranon fracture.

The blood works were within the normal range in all the groups except for higher creatinine kinase and aspartate transaminase levels in the pain groups (Table 2). However, the concentrations of the pain neurotransmitter, SP, of the pain groups, 485.5 ± 250.1 pg/ml, were statistically higher than that of the control group, 116 ± 38.5 pg/ml (P<0.01). Moreover, the SP levels in the serum samples of the dogs with fractures reached 663.3 ± 225 pg/ml, which was significantly higher than those of the dogs with MPL (307.8 ± 105.3 pg/ml), and of the control dogs (116 ± 38.5 pg/ml) (P<0.01) (Fig. 1). Significant differences in SP concentrations were also observed between the dogs with MPL and the control dogs (P<0.01). However, the serum SP levels of one dog in the MPL group overlapped with those of the control dogs, and the levels of two of the

Table 1: Characteristics of the enrolled dogs

Characteristics	Control dogs (n=20)	Patellar luxation (n=10)	Fracture (n=10)
Age	6.7 years	5.1 years	3.4 years
Gender	7 females, 13 males	4 females, 6 males	5 females, 5 males
Breeds	Mal (4), PO (4), CS (4), Mongrel (4), SC (1), ST (1), YT (1), PM (1)	Mal (5), PO (2), YT (2), Mongrel (1)	CS (1), Mal (4), PM (2), PO (1), SC (1), YT (1)

Mal: Maltese, PO: Poodle, CS: Cocker Spaniel, SC: Schnauzer, ST: Shih Tzu, YT: Yorkshire Terrier, and PM: Pomeranian

Table 2: Results of biochemistry evaluations of dogs with patellar luxation and dogs with fractures

Serum chemistry	Patellar luxation (n=10)	Fracture (n=10)	Reference range
AST (U/L)	123 ± 43*	88.6 ± 19*	10-37
T-bilirubin (mg/dL)	0.2 ± 0.1	0.2 ± 0.2	0.1-0.7
Ca (mg/dL)	9.3 ± 0.7	8.6 ± 1.0	9.1-11.7
Cholesterol (mg/dL)	142 ± 43	157 ± 8.1	127-340
CK (CPK) (U/L)	1520 ± 328*	455 ± 234*	25-167
Glucose (mg/dL)	94.8 ± 7.7	107 ± 18	67-147
LDH (U/L)	498 ± 76	171 ± 68.4	65-269
Lipase (U/L)	225 ± 42	60 ± 34.6	5-90
gamma-GTP (U/L)	3.7 ± 2.1	4 ± 2.2	4-25
Total protein (g/dL)	5.4 ± 0.7	5.5 ± 0.5	4.9-7.2
TG (mg/dL)	47.2 ± 27.6	54 ± 8.5	21-116

AST: Aspartate transaminase, Ca: Calcium, CK: Creatine kinase, CPK: Creatine Phosphokinase, LDH: Lactate Dehydrogenase, GTP: Glutamyltransferase, and TG: Triglyceride. * Higher than reference range (P<0.5)

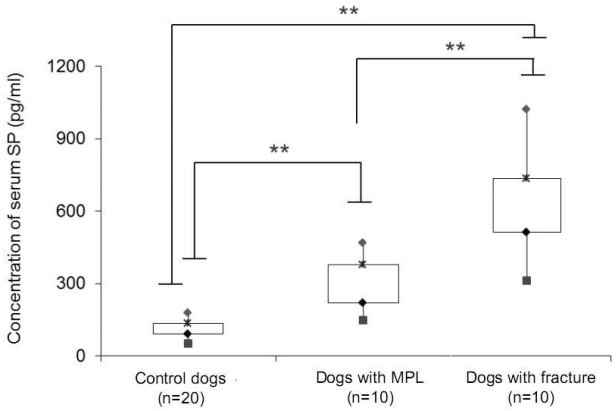

Fig. 1: Serum substance P (SP) concentrations in control dogs, dogs with medial patella luxation (MPL), and dogs with fractures. The serum SP concentrations in dogs with fractures (663.3 ± 225 pg/ml) were significantly higher than those of the control dogs (116 ± 38.5 pg/ml) and the dogs with MPL (307.8 ± 105.3 pg/ml) (** $P<0.01$). In addition, a significant difference in the concentrations of serum SP was also noted between the dogs with MPL and control dogs

dogs with fractures overlapped with the levels of the MPL group.

Discussion

An increased serum SP concentration during a painful event is an expected finding in the field of human medicine (Jang *et al*., 2011). However, in dogs, there have been few attempts to quantify serum SP concentrations associated with the sensation of pain. Orthopedic diseases induce acute pain that usually requires the induction of analgesia (Lisowska *et al*., 2016). In the present study, the two groups of dogs with pain associated with MPL and fractures, respectively, showed significant increased serum SP concentrations, demonstrating a 4-fold elevation compared to those of the control dogs. These findings suggest that SP concentrations are elevated in dogs affected by painful conditions, and that SP could be a potential biomarker for pain assessment in dogs.

In the field of veterinary medicine, previous studies have evaluated and reported on SP concentrations associated with painful conditions in pigs (Ison *et al*., 2016) and cattle (Coetzee *et al*., 2008; Allen *et al*., 2013; Rodriguez *et al*., 2018). In an experimental nerve root compression model in pigs, the SP concentration was elevated in compressed spinal nerve roots compared to in uncompressed controls (Ison *et al*., 2016). In addition, it has been reported that SP concentrations increased after dehorning (Allen *et al*., 2013) and castration (Coetzee *et al*., 2008) in cattle. In addition, in dairy cows with lameness, increased plasma SP concentrations were associated with intensely painful states (Rodriguez *et al*., 2018). However, it was insufficient to differentiate these states from the mildest of painful states, as elevated SP concentrations were only observed in calves with severe pain. In humans, it has been reported that serum SP concentrations were positively correlated with acute pain intensity in patients with rheumatoid arthritis. In this study, we divided dogs into pain groups based on orthopedic diagnoses. Based on the Preemptive Pain Scoring System that estimates the pain level depending on the disease type, the MPL dogs were thought to have mild to moderate pain, and the bone fracture patients were thought to have severe pain. Serum SP concentrations in dogs with fractures were significantly increased compared to in dogs with MPL. These findings support the hypothesis that serum SP levels increase when dogs experience greater pain. However, the SP levels found in one dog in MPL group overlapped with the levels of the control dogs, and the levels found in two dogs of the fracture group overlapped with the levels of MPL group. Therefore, simply measuring SP as an indicator of pain is not enough, and pain assessment needs to be conducted with a combination of behavioral and physiological assessments and by measuring pain biomarkers.

Cortisol and catecholamines have been previously reported to be other biomarkers for pain assessment in dogs. The measurement of cortisol levels has been used as an indicator for increased stress or pain (Srithunyarat *et al*., 2017). However, because the serum cortisol concentration is affected by circadian rhythm and pulsatile secretion (Kemppainen and Sartin, 1984), it has limited potential as a pain biomarker. In addition, it has also been reported that catecholamine is difficult to evaluate as a potential pain biomarker due to its short half-life (Derbyshire and Smith, 1984). Chromogranin A is released with catecholamines when the sympatho-adrenal-medullary system is activated (O'Connor and Bernstein, 1984). In a recent study, the concentration of plasma catestatin, which has an inhibitory role as a negative feedback for the release of catecholamines and chromogranin A, has been proposed as biomarker for pain assessment in dogs (Srithunyarat *et al*., 2016; Srithunyarat *et al*., 2017). The serum catestatin concentrations were significantly lower in dogs with traumatic bone fractures compared to in the healthy control group. However, it has been revealed that catestatin has limited potential to be a biomarker for pain assessment as well, as the correlation between plasma catestatin levels and the pain scores was observed to be very weak, and plasma catestatin concentrations in dogs experiencing pain overlapped to a large degree with the normal ranges in healthy dogs (Srithunyarat *et al*., 2017). In this study, we only evaluated the possibility of a quantitative assessment of pain by comparing the serum SP concentrations between groups of dogs experiencing pain and dogs with no pain. One of the potential limitations of our study was that a pain scoring system, such as the visual analog scale and Glasgow composite measure pain scale, was not applied to assess individual patients. In addition, a limited numbers of dogs were enrolled in this study, and samples were only collected at their initial presentations. A future large-scale study for investigating the correlation between serum SP concentrations and individual pain scores associated with various painful conditions would provide further

information on serum SP as a potential biomarker for pain assessment. In addition, the changes in SP concentrations after dogs have been treated by inducing analgesia also need to be investigated.

In conclusion, the present study provides fundamental data to support the evaluation of serum SP concentration as a potential biomarker for pain assessment. In addition to using pain scoring systems, quantification of serum SP concentrations in dogs affected by painful conditions might provide further information for pain assessment in dogs.

Acknowledgements

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ012843052018)" Rural Development Administration and partially supported by the National Research Foundation (NRF-2013R1A1A2057479), Republic of Korea.

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