MEASUREMENT OF INTERLEUKIN 1β AND INTERLEUKIN 6 IN SYNOVIAL FLUID OF OSTEOARTHRITIC DOGS

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Summary


Osteoarthritis is a degenerative process which involves different structures of the synovial joints. Degradation and repair of the articular cartilage – the most important involved structure – were investigated by measuring interleukin (IL) 1β and IL-6, joint markers of degradation and repair respectively. To this end, five mature large size mixed-breed male dogs were used for this study. Synovial fluid samples were obtained from joints by aseptic arthrocentesis before surgical sectioning of cranial cruciate ligament (CCL) and on days 14, 28, 90 and 180 after CCL transaction. Statistical analysis of the results showed that both cytokines exhibited an increasing pattern. IL-1β showed significant increase after six months and IL-6 showed a non-significant increase. There was no significant correlation between these cytokines during the study. We concluded that although both degradation and repair were in progress in osteoarthritic joints, degradation was predominant over repair when no treatment was performed to slow down the process.

Key words: dog, IL-1β, IL- 6, osteoarthritis, Pond-Nuki (CCL) model, synovial fluid

INTRODUCTION

Osteoarthritis (OA) is a slow progressive disorder of synovial joints and is characterised by an imbalance between synthesis and degeneration of articular cartilage constituents, leading to subsequent destruction of joint cartilage, remodelling of the underlying bone, osteophyte formation, and variable degrees of synovitis (Fernandes et al., 2002). The possible pathogenesis for synovial membrane inflammation includes joint instability or incongruity that induces mechanical damage, which results in the recruitment of several inflammatory mediators that further damage articular tissues (Dupuis & Harari, 1993). Several markers of joint inflammation associated with OA have been identified in humans and dogs and include interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-6, matrix metalloproteinases (MMPs) substance P, prostaglandin E2, and nitric oxide (Westacott & Sharif 1996; Carter et al., 1999; Schmidt-Rohlfing et al., 2002; Maccoux et al., 2007). There is evidence of inflammation in stifle joints with CCLR (Doom et al., 2008). The proinflammatory cytokines IL-1, IL-6, and TNF-α also display many biologic functions in the early phase of joint and cartilage inflammation (Doom et al., 2008). Cytokines in synovial fluid may be derived from synoviocytes, chondrocytes, lymphocytes, and macrophages (Punzi et al., 2002). IL-1 and TNF are
known to play important roles in cartilage metabolism with osteoarthritic changes, as these cytokines promote cartilage matrix degradation and suppress cartilage matrix repair in humans (Tetta et al., 1990; Wood et al., 2005). On the other hand, IL-6 promotes tissue inhibitor of metalloproteinase (TIMP) synthesis and thus may help slow down cartilage matrix degradation in humans (Houssiau et al., 2005). Other researchers investigated biochemical markers in synovial fluid with a variety of diagnostic techniques (Dam et al., 2009; Karsdal et al., 2010; Tourville et al., 2013).

Cranial cruciate ligament rupture (CCLR), one of the most common injuries in dogs is a major cause of OA of the stifle (knee) joint, and is being used as a model in OA studies. There are few reports of alterations in these synovial fluid markers in dogs with OA after CCLR (Venn et al., 2005). Fujita et al. (2006) assessed proinflammatory cytokines activity, MMPs and sulfated glycosaminoglycans in synovial fluid of dogs with CCLR. They showed all the measured data were significantly elevated in stifle joints with CCLR. They also suggested that these inflammatory changes are associated with depletion of proteoglycan from articular cartilage. There are some published researches which have been done on dogs with spontaneous or experimental CCLR (Page et al., 2010; El-Hadi et al., 2012).

There are controversies between researchers regarding advantage or disadvantage of each measured parameter as well as limitations in each study. Since OA is a simultaneous reparative and degradative process, we hypothesised that the activities of these cytokines would be altered during the progression of OA in joints. Therefore, our purpose was to compare IL-1β (as a marker of degradation) and IL-6 (as a marker of regeneration) at different time intervals after OA onset to elucidate degradative versus reparative status of the joints during OA progress, and to compare with the baseline levels of the same cytokines in synovial fluid before OA induction.

MATERIALS AND METHODS

Experimental animals and protocols

Animal selection, all experiments, subsequent care, and the sacrifice procedure all adhered to the same guidelines under supervision of Animal Care Ethics Committee of Shiraz School of Veterinary Medicine (NIH publication NO. 86–23, revised 1985). This study was part of a larger investigation to characterise the development of OA due to experimental stifle joint instability in the dog. Five mature (19±5 months), large (weighing 21.2±2.3 kg), mixed breed, male dogs were used for this study. All dogs were considered healthy based on clinical, orthopaedic, and laboratory findings before surgery as well as normal findings of the stifle joints during arthrotomy. Lateromedial and caudocranial radiographic views were obtained before surgery. Surgeries and sample collections were carried out aseptically. The protocol of anaesthesia, surgical procedures, postoperative care and sacrifice were identical for all animals. During the experiments the animals were housed one per cage and were allowed to move freely 4 h a day in a wide fenced area but were not forced to exercise.

Operative procedure and samplings

The animals were premedicated with acepromazine (0.1 mg/kg IM) and the whole
stifle region was aseptically prepared for surgery. Thirty minutes later, anaesthesia was induced by combination of ketamine (5mg/kg IV) and diazepam (0.2 mg/kg IV). The animals were placed on dorsal recumbency, and the CCL of the left knees were transected through a 4 mm parapatellar stab incision as was previously described for dogs (Pond & Nuki, 1973). Then the incision was sutured in layers. The animals were given appropriate anti-inflammatory drugs for three days post surgery.

**Samples and interleukin assays**

The synovial fluid aspirated from the stifle joints once before surgery (as baseline) and on days 14, 28, 90 and 180. For aspiration of synovial fluid, the medial parapatellar approach was used (Clements, 2006). All synovial samples were centrifuged at 4 °C, at 4000×g for 10 min to separate cells and debris. Supernatants were stored at −70 °C until assay (Fujita et al., 2006).

IL-1β and IL-6 were measured in synovial fluid by commercial canine ELISA Kits (Cusabio Biotech Co., Ltd. China).

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**Statistical analysis**

Significant differences for each measured parameter between different times were evaluated using Repeated Measures Analysis of Variance (ANOVA) and Tukey multiple comparisons as post-hoc test. Pearson’s correlation coefficient was used for determination of the relationship between IL-1 and 6 at different time points. All values were expressed as mean and standard error (SE), and P<0.05 was considered as statistically significant. All data were analysed using computer software GraphPad Prism for windows version 5.01 (GraphPad Software, Inc.).

**RESULTS**

Cranial drawer motion test which is routinely used for diagnosis of CCLR was performed and was positive for all operated joints confirming the full thickness CCLR. There was also mild swelling for three to four days after surgery which subsided afterward and mild weight-bearing lameness for two months after surgery that turned into a

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**Fig. 1.** Changes in synovial IL-1β concentrations (pg/mL) over time in dogs with experimental cranial cruciate ligament transection. Data are shown as mean ± SEM. *P<0.05 vs baseline.

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Statistical analysis revealed that the measured IL-1β values showed an increase over time. However, the changes were not significant until six months post surgery. The measured values of IL-6 showed a non significant increase over time. Both measured cytokines showed an increasing pattern of changes, however, the rate of changes was higher for IL-1β than IL-6 (Figs. 1 & 2). No significant correlation was seen between IL-1β and IL-6 at different time points.

DISCUSSION

Two widely investigated cytokines, IL-1 and IL-6 play important roles in OA. Synovial fluid of osteoarthritic joints is reported to contain a high amount of these two cytokines (Hay et al., 1997). As described previously, IL-1β is degradative and IL-6 is reparative in the metabolism of osteoarthritic articular cartilage. In this study the trends for both cytokines were increased. The changes of IL-1β activity were significant at the end of the six-month period. The changes in the IL-6 activity, despite the increase, were not significant. The changes in these two markers indicate that both degradation and repair are in progress in osteoarthritic joints. As hypothesised, since no treatment was performed in these cases, degradation should be dominant to repair. This was apparent from the significant changes in IL-1β at the end of the study.

Although statistically insignificant, there was a close relationship between these cytokines and the activity of matrix metalloproteinase (MMP) (Houssiau et al., 2005). The MMP family is a group of zinc-dependent endopeptidases. When MMPs are activated, these proteolytic enzymes degrade the extracellular matrix (e.g. collagens and proteoglycans) (Fujita et al., 2006). Although the exact mechanisms of OA in different joints are not fully understood, these measurements help reveal the hidden aspects of the involved mechanisms. There are contradictory results in publications. Some are in accordance (Kaneyama et al., 2002; Fujita et al., 2006) with our findings while others are not (Carter et al., 1999). Several possible factors have been previously described for the contradictory findings (Hay et al., 1997; Fujita et al., 2006).
Increased IL-6 activity in CCLR synovial fluid is consistent with previous reports in dogs with both naturally acquired CCLR and experimentally transected CCL (Hay et al., 1997). IL-6 functions to increase the number of inflammatory cells in synovial tissue, to stimulate the proliferation of chondrocytes, to inhibit proteoglycan production, and to induce the production of TIMP in humans (Fernandes et al., 2002; Houssiau et al., 2005). Ours and other similar results show that joint inflammation could occur and that repair might occur in OA after CCLR because of elevated IL-6 activity; however, significantly increasing IL-1β indicates that without any treatment degradation, predominant repair may take up to six months, at least. Based on the results shown in this study, measuring the synovial fluid IL-1β or IL-6 could be used to monitor the cartilage breakdown or repair status or the efficacy of any surgical or reparative procedures done to the joints.

Adult dogs’ synovial fluid was used for this study. This could be a limitation as joint metabolism may vary between breeds, ages, and body weights. One report (Hay et al., 1997) described a positive relation between IL-6 activity and age and concluded that there was age-related dysregulation of IL-6 production. It may be desirable for future studies to use at least age matched controls. There was no correlation between these markers. One explanation is that at some stages cartilage degradation is prominent and in others physiologic repair might occur. Because both processes are ongoing, no one showed a significant difference to the other until degradation was far beyond the repair. It is also possible that other cytokines and mediators may be associated with the regulation of these markers.

Cartilage breakdown markers are mainly diffused in synovial fluid and later via lymphatics distributed into bloodstream. In the related synovial fluid it is concentrated and measurable, whereas in blood, it is diluted so that measurement of extremely low concentrations is rather difficult. This could be one explanation why we got high concentration of the measured marker while the changes where insignificant in serum.

Since no obvious changes were seen in these markers between the first two weeks of the study to the other time points especially week four, we concluded that the changes were not related to the arthroscopy and were related to the time passed from OA, indicating that more chronic stages had more ILs alterations.

In conclusion, this study has found that measuring synovial cytokines reflects the degradative or reparative status of the joints. Further, while OA is in progress with no treatment, although both cytokines increased, degradation (IL-1β) predominated over physiologic repair (IL-6). Measurement of these markers during therapy may show effects on the cartilage breakdown or repair.

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