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Original Research

Interleukin-1 Receptor Antagonist and Interleukin-1 Beta Levels in Equine Synovial Fluid of Normal and Osteoarthritic Joints: Influence of Anatomic Joint Location and Repeated Arthrocentesis

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ABSTRACT

This study was aimed to evaluate the concentrations of interleukin-1 receptor antagonist (IL-1ra) and interleukin-1 beta (IL-1β) in normal and osteoarthritic (OA) joints as well as the influence of joint location and arthrocentesis on these concentrations. Interleukin-1 receptor antagonist and IL-1β levels were determined in the synovial fluid (SF) of 18 normal and 18 OA joints. In all normal joints, arthrocentesis was repeated after 1 hour. No significant difference of SF IL-1ra and IL-1β levels between metacarpophalangeal/metatarsophalangeal, radiocarpal, and talocrural joints was observed. There was no significant change in SF IL-1ra and IL-1β levels between first and second arthrocentesis detectable. Synovial fluid IL-1ra and IL-1β levels were significantly increased in OA joints compared to normal joints. Synovial fluid WBC count and protein concentration were not significant different between normal and OA joints. Synovial fluid WBC count and protein concentration as well as IL-1ra and IL-1β concentration were positive correlated. The anatomic location of high motion joints seems to have no influence on SF IL-1ra and IL-1β levels. Arthrocentesis did not increase SF IL-1ra and IL-1β levels within 1 hour after joint puncture. Increased SF IL-1ra, IL-1β, and protein concentrations as well as WBC counts seem to be indicators of joint inflammation, but on their own are not allowing an exact differentiation between healthy and mild OA joints due to great value ranges and value overlap. Yet it has to be further investigated if in combination with other biomarkers, a clearer differentiation of pathologic processes in the joint can be made.

1. Introduction

Current osteoarthritis (OA) research focuses on the elucidation of the biochemical processes in OA pathology and the development of disease-modifying therapies.

Earlier studies assumed that a disproportion in the ratio between the proinflammatory cytokine interleukin-1 beta (IL-1β) and its natural antagonist interleukin-1 receptor antagonist (IL-1ra), with especially a surplus of IL-1β and a relative deficiency of IL-1ra mainly contributes to the progression of OA and therefore might play a substantial role for the outcome of regenerative therapies [1,2].

Consequently, one regenerative therapeutic approach is to counteract the degenerative effects of IL-1β by substitution of IL-1ra using autologous conditioned serum (ACS)

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[3,4], platelet rich plasma [5–7], or gene therapy [8–10]. Detailed information of the physiological concentrations of IL-1ra and IL-1β in normal joints compared to OA joints and in joints of different anatomic locations as well as the influence of closely repeated arthrocentesis on these interleukin concentrations would be of great importance for a better understanding of the treatment application as well as its evaluation and improvement. Current knowledge in this field is very limited in both humans and horses.

The aim of this study was to investigate the influence of anatomic variations and closely repeated arthrocentesis on IL-1ra and IL-1β levels in the synovial fluid (SF) of normal joints and to determine the IL-1ra, IL-1β, and protein concentrations as well as the white blood cell (WBC) count in the SF of normal and OA equine joints, using equine-specific antibody enzyme-linked immunosorbent assays (ELISAs).

2. Materials and Methods

2.1. Animals

Samples of SF were obtained from 24 mature horses of various breeds, sexes, and ages (10 years ± 4.7 years). Six of these were healthy horses (13.8 years ± 3.1 years) which served as a control group. They were owned by the department and were used for clinical teaching of undergraduate students in noninvasive procedures, such as clinical examinations or bandaging. Inclusion criteria for the control group were absence of lameness in walk and trot, negative flexion tests, and no radiographic abnormalities in the joints of all four limbs. Eighteen horses (8.5 years ± 4.5 years) were presented to the clinic due to an orthopedic problem associated with joint disease (OA group). Diagnosis of OA was based on a comprehensive orthopedic examination, including evaluation of lameness, joint distension, pain on joint flexion, and perineural and/or intraarticular anesthesia. If intraarticular anesthesia was necessary, SF samples were taken before application of the local anesthetic. Each horse had radiographic examination and diagnostic arthroscopy of the affected joints. All horses were suffering of mild chronic OA with a duration of joint swelling for minimum 3 months and lameness of minimum 2 weeks. The joint disease of horses in the OA group was graded by the classification system used by Ehrle and Lischer [11] based on radiographic and arthroscopic findings (Table 1).

Informed owner consent was obtained, and sample collection was performed following approval of the institutional ethics committee of the mask for peer review.

Each of the two groups (control and OA) included six metacarpophalangeal/metatarsophalangeal joints, six radiocarpal joints, and six talocrural joints. None of these joints had received synovial analgesia or intraarticular medication within 1 month before SF aspiration. Horses selected for this study were subjected to a thorough clinical examination and a hematological profile (cytologic evaluation, total WBC count, and hematocrit). Any horse displaying clinical symptoms unrelated to OA was excluded from the study.

Table 1
Classification of 18 horses with joint disease divided by anatomic location of the joint using radiographic, inflammatory, and cartilage degeneration scores.

Group	Radiographic Score	Inflammatory Score	Cartilage Degeneration Score	Initial Pathology
MCJ/MTJ (n = 6)	3.33 ± 1.5	1.33 ± 0.82	0.67 ± 0.52	OCD (six horses)
RCJ (n = 6)	6.67 ± 2.73	3 ± 1.55	1.17 ± 0.75	OCD (four horses) and OA (two horses)
TCJ (n = 6)	4.78 ± 2.53	2 ± 1.28	0.83 ± 0.62	OCD (six horses)

Abbreviations: MCJ, metacarpophalangeal joint; MTJ, metatarsophalangeal joint; OA, osteoarthritis; OCD, osteochondrosis dissecans; RCJ, radiocarpal joint; TCJ, talocrural joint.
All horses were suffering of mild chronic OA with radiographic scores of 0–10, inflammatory scores of 0–5, and cartilage degeneration scores of 0–2.

2.2. Experimental Design and Sample Collection

OA group: All SF samples from clinical cases were taken either during diagnostic procedures or before arthroscopy.

Control group: SF samples of one metacarpophalangeal/metatarsophalangeal, one radiocarpal, and one talocrural joint were obtained from each healthy horse. Each arthrocentesis was repeated after 1 hour.

All SF samples were taken using a strictly aseptic technique. After aspiration, samples were placed in tubes containing ethylenediaminetetraacetic acid, centrifuged at 4,000*g* for 10 minutes at 4°C, chilled within 20 minutes to −80°C and stored until biochemical analysis.

2.3. IL-1Ra and IL-1β Quantification

For the quantitative determination of IL-1ra and IL-1β in SF, commercially available ELISA kits using specific antibodies against equine IL-1Ra (Raybiotech Inc, Norcross, GA) and IL-1β (Cloud-Clone Corp, Houston, TX) were used.

Both kits were sandwich enzyme immunoassays and were processed according to the manufacturer's instructions. The kits were validated for use on equine SF using standard parallel and serial dilutions. Validation assays generated consistent results in intraassay and interassay comparisons. The intraassay coefficient of variation was under 10%, and the interassay coefficients were 12% for IL-1ra and 13.5% for IL-1β. Each sample was measured in duplicate. Samples were undiluted for measuring IL-1β and were diluted 1:5 with sample diluent before IL-1ra evaluation.

2.4. WBC Counts and Protein Quantification

Total WBC counts were performed on all SF samples using automated cell counter (Coulter counter T 840, UK). White blood cell counts were expressed in cells/L. Protein quantification was performed using the Bio-Rad Protein Assay (BioRad Laboratories GmbH, Munich) based on the method of Bradford [12] in accordance with the manufacturer's protocol.

2.5. Statistical Analysis

Statistical analysis was performed using the SPSS statistics package (version 21.0; SPSS, IBM Corporation, Armonk, NY). Data of radiographic, inflammatory, and cartilage degeneration scores were normally distributed. The rest of the data were not normally distributed (Kolmogorov–Smirnov test), and a normalization of data was not possible. For comparison of IL-1ra and IL-1β concentrations in joints of different anatomic locations, a Kruskal–Wallis test was used. Changes in SF concentrations of IL-1ra and IL-1β between the first and second joint puncture were analyzed using a Wilcoxon test. The differences in SF interleukin and protein concentrations as well as serum and SF WBC counts between normal and OA horses were analyzed using a Mann–Whitney *U* test. Spearman's coefficient of correlation was calculated to demonstrate any correlation between parameters. A *P* value below .05 was considered statistically significant.

3. Results

3.1. High Motion Joints of Different Anatomic Locations

Interleukin-1 beta concentrations in the SF of normal joints consistently remained at the detection limit (15.6 pg/mL). For statistical analysis, all values below the detection limit were set to 15.6 pg/mL.

The SF IL-1ra and IL-1β levels in metacarpophalangeal/metatarsophalangeal, radiocarpal, and talocrural joints were not significantly different ($P = .854$, Fig. 1). Therefore, data obtained from all joints were combined to determine the influence of repeated arthrocentesis and the difference between normal and OA joints.

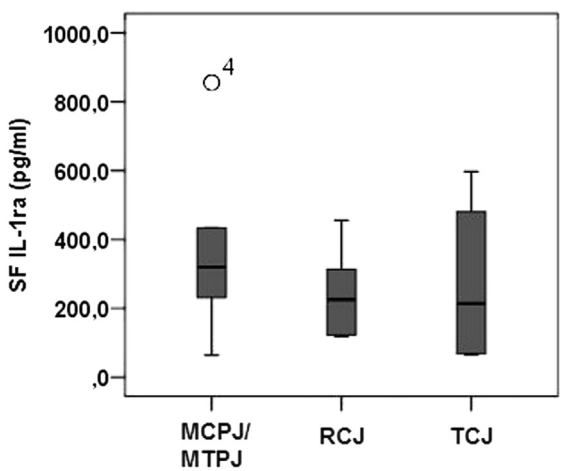

Fig. 1. IL-1ra concentrations in metacarpophalangeal/metatarsophalangeal (MCPJ/MTPJ), radiocarpal (RCJ), and talocrural (TCJ) joints of normal horses. Boxplots represent the interquartile ranges (IQRs) of n = 6. Black lines represent the medians. Whiskers represent values outside the IQR. ° indicates an extreme value. IL-1ra, interleukin-1 receptor antagonist; SF, synovial fluid.

3.2. Repeated Arthrocentesis

In normal joints, no statistically significant changes in IL-1ra ($P = .231$) and IL-1β ($P = .980$) levels between the first and second arthrocentesis were detected (Fig. 2).

3.3. Normal and OA Joints

Synovial fluid IL-1ra ($P = .047$) and IL-1β ($P < .001$) concentrations in OA joints were significant higher than in normal joints (Fig. 3, Table 2).

There was no statistically significant difference in SF WBC and protein concentration and serum WBC count between normal and OA horses (Fig. 4, Table 2).

Synovial fluid protein concentration was positive correlated with the SF WBC count (correl. coeff.: 0,67, $P = .01$). In addition, SF IL-1ra and IL-1β concentrations showed a positive correlation trend (correl. coeff.: 0,33; $P = 0{,}05$). No correlation was detectable between SF cytokine and protein as well as WBC concentrations.

4. Discussion

Equine studies on OA pathology and management have been performed to a large extent on an induced OA model in the middle carpal joint [13,14] or different joint types with natural occurring OA [15]. Trumble et al [16] detected a significant difference in bone alkaline phosphatase concentration in the SF between carpal and metacarpophalangeal/metatarsophalangeal joints. Considering the different types and disease stages of natural occurring OA and the resulting wide ranges of cytokine levels in OA joints, the demarcation of equal groups of horses suffering from OA for scientific analysis is difficult and includes many bias. Consequently, further elucidation of the SF composition of normal joints might help to understand more of the OA pathology. The present study is showing no difference of SF IL-1β and IL-1ra concentrations between high motion

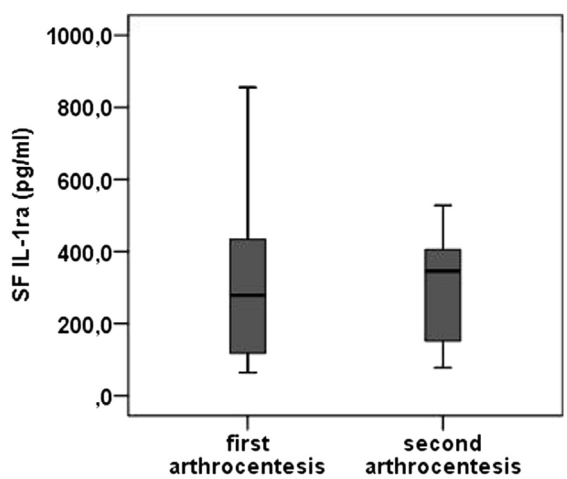

Fig. 2. IL-1ra concentrations after first and second arthrocentesis. Boxplots represent the interquartile range (IQR) of n = 18. Black lines represent the medians. Whiskers represent values outside the IQR. IL-1ra, interleukin-1 receptor antagonist; SF, synovial fluid.

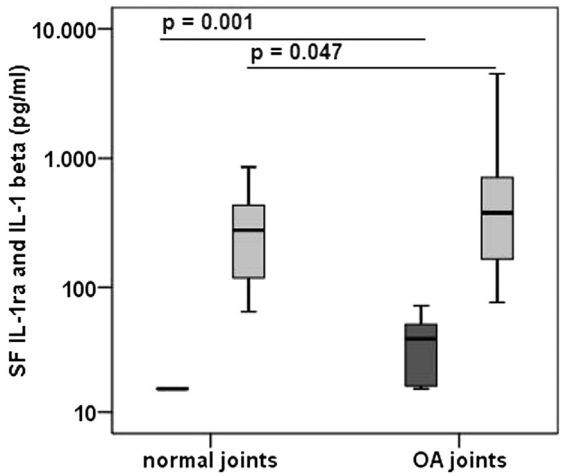

Fig. 3. IL-1ra (light gray bars) and IL-1β (dark gray bars) concentrations in SF of normal joints and joints suffering from OA. Boxplots represent interquartile ranges (IQRs) of n = 18. Black lines represent medians. Whiskers represent values outside the IQR. The lines connecting graphs represent significant differences ($P \leq .05$). IL-1β, interleukin-1 beta; IL-1ra, interleukin-1 receptor antagonist; OA, osteoarthritis; SF, synovial fluid.

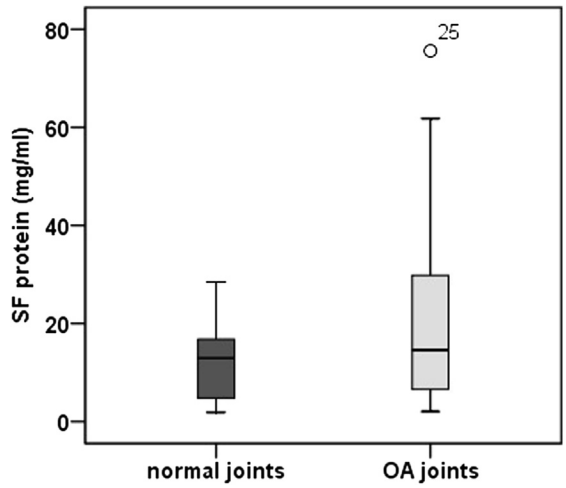

Fig. 4. Protein concentrations in normal joints and OA joints. Boxplots represent the interquartile range (IQR) of n = 18. Black lines represent the medians. Whiskers represent values outside the IQR. °indicates an extreme value. OA, osteoarthritis; SF, synovial fluid.

joints of three different anatomic locations in normal horses. These data suggest a similar SF IL-1β and IL-1ra concentration in metacarpophalangeal/metatarsophalangeal, radiocarpal, and talocrural joints, and thus, a possible transferability of investigations made in one of these joints to high motion joints in other anatomic locations. Yet a great bias of this study is the small sample size which does not allow a secure statement. Therefore, these findings need to be revaluated in future studies with greater sample size, and the transferability to other high and low motion joints should be clarified.

The microlesions caused by a needle puncture might already promote increases in SF IL-1ra and IL-1β levels. Previous studies showed that increases in several other biomarkers were significantly associated with repeated arthrocentesis [17–19]. In those studies, the first SF follow-up samples were taken 12 hours [17] and 14 days [18,19] after the first arthrocentesis. Different studies have suggested very short half-life periods (i.e., 4–5 hours) for IL-1ra and IL-1β [20–22]. Interleukin-1 beta in particular is known to be one of the major cytokines that increases rapidly during inflammatory insults. Thus, a timely more frequent observation of the IL-1ra concentration in SF after different joint treatments as ACS or platelet rich plasma injection as well as intraarticular gene delivery could be revealing in future studies. The present study used a sample interval of 1 hour between the first and second arthrocentesis and could detect no statistically significant difference in SF IL-1ra and IL-1β concentrations within 1 hour after joint puncture of normal joints. It might be reasonably assumed that IL-1ra and IL-1β levels will increase at later time points due to induced inflammation by arthrocentesis. In retrospect, further SF samples within the first 48 hours after arthrocentesis could have been able to clarify this question and displays another limitation of the study. Yet thinking in future studies, the gained knowledge already allows a closer sampling after joint treatment and a better judgment of results within the first hour after arthrocentesis because if there is an inflammatory insult by arthrocentesis in form of increasing IL-1ra and IL-1beta levels, these are not detectable within the first hour after arthrocentesis. A parallel investigation in OA joints would have given information about the transferability of these findings to inflamed joints, yet it was not possible due to owner consent. Martel-Pelletier et al [23] found that the number of IL-1 receptors is twofold higher in OA than in normal chondrocytes and that cells in OA joints need less IL-1 receptor occupancy to induce the release of proinflammatory cytokines than in normal joints. Thus, a triggering of the

Table 2
Median, mean ± standard deviation (SD), and the 95% confidence interval (CI) of the median of synovial fluid (SF) and serum concentrations of IL-1ra, IL-1β, protein, and white blood cells (WBCs).

Concentrations	n	Normal Horses			OA Horses		
		Median	Mean ± SD	95% CI of Median	Median	Mean ± SD	95% CI of Median
SF IL-1ra (pg/mL)	18	278.3	295.9 ± 214.9	120.8–403.3	415.8	707.5 ± 1,011.9	241.5–654.4
SF IL-1β (pg/mL)	18	≤15.6	≤15.6	—	39.7	38.0 ± 20.6	17.6–50.3
SF protein (mg/mL)	18	13	12.5 ± 8.3	6.4–16.7	14.6	22.8 ± 21.7	10.1–26.1
SF WBC count ($\times 10^6$ cells/L)	18	0.1	0.1 ± 0.0	0.1–0.1	0.1	0.15 ± 0.09	0.11–0.15
Serum WBC count ($\times 10^3$ cells/L)	18	5	5.56 ± 1.3	4.7–5.7	5.6	5.9 ± 1.1	5.0–6.6

Abbreviations: IL-1β, interleukin-1 beta; IL-1ra, interleukin-1 receptor antagonist; OA, osteoarthritis.

joint inflammation due to arthrocentesis in OA joints might be very likely and need to be investigated.

Conforming to previous studies, in the present study, IL-1β levels in the SF of normal horses uniformly stayed at the detection limit (15.6 pg/mL). Kamm et al [24] presumed that IL-1β levels in SF lay below 1 ng/mL and are not detectable by currently commercially available antibody ELISAs. Dinarello [25] reported that with the exception of skin keratocytes, no significant amounts of IL-1β were observed in most other healthy cells. Furthermore, they discovered that IL-1β production increased dramatically in a variety of cells in response to inflammatory agents. A result that was also reflected in the IL-1β levels in the SF of OA horses in the present study, which were significantly higher than those in normal horses and in agreement with measured values in OA joints in recent equine and human studies [11,26,27].

In addition, IL-1ra levels in the SF of OA joints were, conforming with previous equine studies [11,26], statistical significantly increased compared to normal joints. Furthermore, we could detect a moderate positive correlation between SF IL-1β and IL-1ra. These results support the assumption of a relative deficiency of IL-1ra to IL-1β in chronic OA joints to be one of the major contributing factor to the progression of OA [1,2]. The body seems to counter regulate the IL-1β increase during OA pathology by a simultaneous increase of IL-1ra and tries to uphold the innate IL-1ra/IL-1β ratio. The IL-1ra concentration in OA joints in the present study exceeded the IL-1β concentration by fourfold to 55-fold. Yet, despite this simultaneous increase, the body seems to be unable to prevent the progress of OA pathology. As already assumed by Ehrle et al [11], this could be explained by the necessity of a much larger excess of IL-1ra over IL-1β (100–2000 times higher) to inhibit IL-1β activity [28], which in turn might find its explanation in the aforementioned study results of Martel-Pelletier et al [23] who found increased IL-1 receptor density in OA chondrocytes and synovial fibroblasts compared to normal joint tissue. Richette et al [29] found a significant increased IL-1ra/IL-1β ratio in human OA knee joints compared to rheumatoid knee joints, yet there was no comparison done to normal joints. Unfortunately, it was not possible to measure the exact amounts of SF IL-1β concentrations in the present study what would have allowed to calculate a ratio between IL-1ra and IL-1β, which would have been of great interest and is another major limitation of the study.

Comparing the SF WBC count and protein concentration between both groups, no statistical difference could be detected which is in agreement with previous equine studies [30,31] who found higher SF WBC counts and protein levels in septic arthritis but not in OA joints compared to normal joints. As Fietz et al [30], the present study also could detect a positive correlation between both parameters. Yet, SF WBC count and protein concentration showed no correlation with SF IL-1ra and IL-1β levels. These findings seem to be contradictory because increased SF WBC and protein level as well as SF IL-1ra and IL-1β levels seem to indicate joint inflammation. Different human and equine in vitro studies showed that biologic responses of joint tissues to inflammatory insults are different [5–7,27], which could indicate that the kind of cytokines and the amount that is produced in OA joints depends much more on the kind of joint tissue that is mainly injured. Thus, the importance of an exact defining of equal patient groups especially using patients suffering from natural occurring OA and recruiting of much greater sample sizes, due to wide value ranges, will be of enormous importance in future studies and are major limitations in the present study.

4.1. Conclusions

The anatomic location of high motion joints seems to have no influence on SF IL-1ra and IL-1β levels. Arthrocentesis did not increase SF IL-1ra and IL-1β levels within 1 hour after joint puncture. Increased SF IL-1ra, IL-1β, and protein concentrations as well as WBC counts seem to be indicators of joint inflammation, but on their own are not allowing an exact differentiation between healthy and mild OA joints due to great value ranges and value overlap. Yet, it has to be further investigated if in combination with other biomarkers, a clearer differentiation of pathologic processes in the joint can be made.

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