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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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A B S T R A C T

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.
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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.

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