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Hyaluronic acid concentrations in synovial fluid of dogs with different stages of osteoarthritis

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ABSTRACT

To compare hyaluronic acid (HA) concentrations measured in synovial fluid (SF) of joints with different stages of canine secondary osteoarthritis (OA), clinical-orthopedic, radiographic, macroscopic intra-operative and SF findings of 49 joints were assessed. The sum of single findings was correlated to HA concentrations measured by a commercially available ELISA. Joints were categorized into three OA-groups: non-osteoarthritic, mildly osteoarthritic, and severely osteoarthritic. A significant negative correlation was found between severity of OA and HA concentrations ($r = -0.696$; $P < 0.001$). Median values of HA concentrations decreased with increasing severity of the disease. Statistically significant differences in HA concentrations were observed between the OA-groups ($P < 0.001$). Due to overlapping values between groups, it was concluded that synovial HA concentrations may only indicate a trend of osteoarthritic disease activity, but is not suitable for staging the disease.

1. Introduction

OA is a common degenerative joint disease affecting dogs of all ages, breeds and sexes. Although different primary lesions may lead to the development of OA, the molecular pathophysiology of the disease is thought to be uniform. Many factors involved in osteoarthritic processes are not fully understood yet, but several studies showed the role of inflammation in the onset and progression of OA (Pelletier et al., 2001; Hegemann et al., 2005; Pearle et al., 2007; Doom et al., 2008).

The most common causes for development of secondary OA are hereditary disorders such as osteochondrosis (OC), fragmented coronoid process (FCP) and patellar luxation (PL) or acquired diseases like anterior cruciate ligament rupture (ACLR). The diagnosis is often based on clinical and radiographic findings. Unfortunately, radiographic changes occur as a result of previous osteoarthritic events, and therefore, radiographs are not suitable to assess the current disease activity or progression (Gordon et al., 2003; Akerblom and Sjöström, 2007; Goldhammer et al., 2010). More precise diagnostic techniques like computed tomography or magnetic resonance imaging are increasingly available, but their application is often limited by considerable expense. Cost-effective methods for early diagnosis, staging and monitoring of the course of the disease would be of great benefit in the treatment of OA. Several clinical and experimental studies on different biochemical markers of joint diseases in dogs have been conducted so far (Fox and Cook, 2001; Garvican et al., 2010a,b). Although promising results were presented, specific markers are not yet available for routine application in small animal practice.

HA is a glycosaminoglycan synthesized by chondrocytes and synovial fibroblasts (Iwanaga et al., 2000; Archer and Francis-West, 2003). In the joint, HA is a major component of SF and the extracellular matrix of cartilage (Fraser et al., 1997). Among other purposes, HA serves as lubricant and plays a major role in shock absorption and distribution of mechanical forces impacting the joint. Moreover, HA inhibits the migration of inflammatory cells (Forrester and Wilkinson, 1981; Partsch et al., 1989) as well as cytokine-induced production of matrix metalloproteinases and other pro-inflammatory mediators (Wang et al., 2006; Santangelo et al., 2007; Waddell et al., 2007) and has analgetic effects by influencing intra-articular pain receptors (Pozo et al., 1997; Gomis et al., 2007). In osteoarthritic joints, the concentration and molecular weight of HA are decreased. These observations were related to fragmentation and altered synthesis of HA (Prehm, 1990; Henderson et al., 1991; Kuroki et al., 2002).

Only a few studies investigated the use of HA concentrations in SF as a biomarker of canine OA. Dogs with naturally occurring OA showed lower HA concentrations in SF than controls (Arican et al., 1994; Venable et al., 2008). The results were variable in experimentally induced OA: compared to baseline values the HA concentrations were significantly decreased over 3 months (Budsberg

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et al., 2006), whereas another study reported no significant differences at 12 weeks after surgery when compared to the untreated contralateral joint (Venable et al., 2008).

This study focuses on HA concentrations in SF of dogs with naturally occurring secondary OA under clinical conditions. We hypothesized that HA concentrations are associated with the severity of the disease. The objectives were to (1) assess OA in dogs by a combination of physical, radiographic and macroscopic examinations, (2) proof the correlation of HA with the severity of the disease, and (3) explore whether HA concentrations differ between dogs with no, mild and severe OA.

2. Materials and methods

2.1. Study population

SF samples of 41 osteoarthritic joints belonging to 40 dogs were enrolled in this study. The dogs were presented to the Small Animal Clinic of the Freie Universität Berlin with secondary OA due to various primary joint diseases. All dogs underwent surgery for treatment of the underlying joint disease. Based on history, physical examination, complete blood count and plasma biochemical profile, dogs with evidence of systemic or organic diseases or treated with steroidal or non-steroidal anti-inflammatory drugs within 14 days prior to surgery were excluded. Dogs had to be over 8 months of age.

Eight joints of seven additional dogs had no osteoarthritic lesions and SF samples served as controls representing healthy joints. The dogs were either euthanized for non-orthopedic reasons or underwent arthrocentesis of multiple joints due to comparison of SF nucleated cell counts between affected and non-affected joints for diagnostic reasons. In the latter, the remaining SF was used for HA quantification. In all controls, joint pathology was ruled out by clinical-orthopedic, radiographic and/or macroscopic evaluation of the joint and synovial fluid analysis.

Twenty-one different breeds, mainly large breeds like Labrador Retriever ($n = 6$), Rottweiler ($n = 5$), Boxer ($n = 4$) and large mixed-breed ($n = 4$), were present. Twenty-nine dogs were male, with seven of them castrated and 18 dogs were female, with six of them spayed. The 40 dogs with joint disease had a median age of 47.5 months (range 9–158 months) and the seven non-osteoarthritic dogs had a median age of 75 months (range 9–148 months). The median weight of the osteoarthritic dogs was 39 kg (range 12–65); the median weight of the control dogs was 36 kg (range 29–65 kg).

Of 41 affected joints, OA was seen in 29 stifle joints ($n = 28$ ACLR, $n = 1$ PL), seven elbow joints ($n = 4$ elbow dysplasia, $n = 2$ OC, $n = 1$ FCP) and five shoulder joints ($n = 4$ OC, $n = 1$ bicipital tendinopathy). SF serving as healthy control was taken from two shoulder joints, two elbow joints and four stifle joints.

All clinical examinations were part of the routine evaluation of orthopedic patients at our institution. SF samples were collected during surgery without intervening with the animals' welfare. Therefore, approval of the animal care and use committee was not required.

2.2. Evaluation of osteoarthritis

The clinical stage of joint disease was assessed by evaluation of lameness, pain, joint effusion and crepitation. Therefore, modifications of previously described scoring systems, based on ordinal scales with predefined descriptions for each grade were used (Bui and Bierer, 2001; Peterson and Keefe, 2004; Hanson et al., 2006; Hazewinkel et al., 2008). Lameness was assessed while the dog was walking and trotting. Joint effusion of the shoulder cannot be palpated. The elbow joint was examined while the dog was standing and effusion was assessed between the lateral epicondyle of humerus and the olecranon. For examination of the stifle joint, with special regard to the patellar ligament, the dog was standing and also placed in lateral recumbency (Table 1).

Radiographs of the affected joints were performed in mediolateral and, in case of elbow and stifle joints, also craniocaudal projections. Osteoarthritic lesions of the shoulder joint were graded by the use of modifications of a previously described scoring system (Akerblom and Sjöström, 2007). The radiographs of the elbow joint were evaluated as previously described (Flückiger, 2010). Radiographic evidence of OA in the stifle joint was evaluated according to Brunnberg (1987) (Table 2).

During surgery, alterations of the joint capsule, synovial membrane and cartilage were assessed by use of modified ordinal scales with pre-defined descriptions for each grade (Loeuille et al., 2005; Cook et al., 2010) (Table 3).

SF was aspirated in the anesthetized patient prior to opening of the joint capsule or insertion of arthroscopic instruments with a sterile 20 G needle and attached syringe. The viscosity of the aspirated SF was evaluated subjectively as described by Fernandes (2008) (Table 4). SF samples were centrifuged at 200*g* for 10 min. Supernatants were stored at −80 °C until assayed.

The sum of each single grade identified with physical, radiographic and macroscopic examination indicates a total OA-score (0–25). Additionally, the joints were classified in three groups by the following definition: total score = 0: non-osteoarthritic, total score = 1–8 or macroscopic or radiographic findings graded $\leqslant 2$: mild osteoarthritic, and total score = 9–25 or macroscopic or radiographic findings graded >2: severe osteoarthritic.

2.3. Hyaluronic acid assay

For determining HA concentrations in canine SF samples, a commercially available ELISA kit (TECO Hyaluronic Acid, TECOmedical AG) was used. The assay is based on a HA-specific binding protein, which is not species-specific. Different molecular weights are detected. SF samples were diluted 1:5000 in diluent. Further processing and analysis of the samples were carried out according to the manufacturer's instructions. All samples were run in duplicates. The susceptibility of errors related to high dilution and high viscosity of SF samples was determined by repeated dilution of three samples and calculation of variations. Inter- and intra-assay precision were determined by a series of five measurements. SF concentrations of HA are reported in μg/mL.

2.4. Statistical analysis

The data was analyzed using the PASW Statistics 18.0 (SPSS Inc.) software. Results are presented as maximum, minimum and median. The coefficient of variation (CV%) was calculated for assessing inter- and intra-assay precision and susceptibility to errors due to sample dilution. Correlations were evaluated using Pearson's correlation coefficient (r). To determine differences in HA concentrations between groups the non-parametric one-factor Analysis of Variance (ANOVA) by either Kruskal–Wallis or Mann–Whitney-*U* test was performed. Values of $P < 0.05$ were considered significant. In the case of significant differences, a paired comparison with Bonferroni-correction for multiple testing was performed.

3. Results

No statistical significant differences were seen in age ($P = 0.752$) or body weight ($P = 0.614$) between dogs with OA and dogs whose joints served as healthy controls. For the HA