Intra-articular injections in the management of canine osteoarthritis

A review of the literature

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Tiivistelmä/Referat – Abstract

The amount of information available on the use of intra-articular medications in the management of canine osteoarthritis is scarce. The purpose of the review was to investigate whether the scientific evidence on the use of intra-articular medications in veterinary and human medicine is strong enough to justify their use in the management of canine osteoarthritis.

Corticosteroids and hyaluronic acid are the most commonly used intra-articular medications both in human and veterinary medicine. Corticosteroids are used because of their ability to suppress inflammation effectively. Long-acting corticosteroid preparations, such as methylprednisolone acetate and triamcinolone acetonide, are preferred for intra-articular use. In Finland methylprednisolone acetate is registered for dogs at a dose of 20 mg given in a large joint cavity. The approved dose of triamcinolone acetonide in the United States is 1,0-3,0 mg.

The scientific evidence on the efficacy of intra-articular corticosteroids in the management of osteoarthritis is stronger in humans than in dogs. In humans they are mainly used in the acute inflammatory disease exacerbations. In horses corticosteroids are often administered together with hyaluronic acid.

Hyaluronic acid is administered to restore the synovial fluid elastoviscosity and to reduce the pain caused by joint movement. Other potential effects are also being investigated. Hyaluronic acid preparations on the market in Finland or in the United States are not approved for intra-articular use in dogs. The dose of hyaluronic acid in the studies in dogs has ranged from 10 to 20 mg.

The scientific proof of efficacy of hyaluronic acid is not strong in veterinary or human medicine. In human medicine the pain relieving-effect of hyaluronic acid has been suggested to be longer-lasting than that of corticosteroids. However, corticosteroids are preferred in states of acute inflammation. In horses hyaluronic acid is only rarely used as the only intra-articular medication.

Other options for the intra-articular therapy of osteoarthritis include the mesenchymal stem cells, autologous conditioned serum, platelet-rich plasma and botulinum neurotoxin. Some of these are already in clinical use.

None of the reviewed medications has a strong scientific evidence of efficacy. However, as the risks associated with intra-articular medications are in general rather small, intra-articular medications might in certain cases be worth trying. The review can be used when planning intra-articular corticosteroid or hyaluronic acid therapy for a canine patient.

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Osteoarthritis, intra-articular, dog, nivelrikko, koira, nivelinjektio, nivelensisäinen

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Tiivistelmä/Referat – Abstract

Tietoa nivelensisäisten lääkitysten käytöstä koiran nivelrikon hoidossa on tarjolla vain vähän. Kirjallisuuskatsauksen tavoitteena oli selvittää, onko tieteellinen näyttö nivelinjektioiden tehosta nivelrikon hoidossa eläin- ja ihmislääketieteessä tarpeeksi vankkaa, jotta nivelinjektioiden käytön koiran nivelrikon hoidossa voidaan katsoa olevan tieteellisesti perusteltua.

Eläin- ja ihmislääketieteessä yleisimpiä nivelinjektioissa käytettyjä lääkevalmisteita ovat kortikosteroidit ja hyaluronihappo. Kortikosteroidien käyttö perustuu niiden kykyyn vaimentaa tulehdusvastetta tehokkaasti. Pitkävaikutteiset kortikosteroidivalmisteet, kuten metyyliprednisoloniasetaatti ja triamsinoloniasetaatti, ovat yleisimmin nivelinjektioissa käytettäviä kortikosteroideja. Näistä metyyliprednisoloniasetaatti on Suomessa rekisteröity koiralle nivelensisäisesti käytettäväksi annoksella 20 mg suureen niveltilaan. Yhdysvaltojen Food and Drug Administration (FDA) on rekisteröinyt myös triamsoniloniasetaatin koiralle nivelensisäisesti annosteltavaksi annoksella 1-3 mg.

Kortikosteroidien tehosta nivelrikon hoidossa on ihmisillä enemmän näyttöä kuin koirilla. Ihmisillä kortikosteroideja käytetään yleisimmin nivelrikon taudinkulkuun usein kuuluvissa akuuteissa tulehdusvaiheissa. Hevosilla nivelensisäisiin kortikosteroidi-injektioihin liitetään usein hyaluronihappoinjektio.

Hyaluronihappoinjektioilla pyritään palauttamaan nivelnesteen elastoviskositeettia ja täten vähentämään nivelen liikkeen aiheuttamaa kipua. Myös muita hyaluronihapon mahdollisia vaikutuksia tutkitaan. Hyaluronihapon tehosta ei ole vankkaa tieteellistä näyttöä eläin- tai ihmislääketieteessä. Ihmisillä tehtyjen tutkimusten perusteella hyaluronihapon kipua lievittävä vaikutus saattaa olla pidempi kuin kortikosteroidien. Kortikosteroidit ovat kuitenkin yleisesti ensisijainen injektiolääkitysvaihtoehto nivelrikon taudinkuvaan kuuluvissa akuuteissa tulehdusvaiheissa. Hevosella hyaluronihappoinjektioita käytetään vain harvoin ainoana nivelensisäisenä lääkityksenä.

Muita mahdollisia vaihtoehtoja ovat esimerkiksi mesenkymaaliset kantasolut, potilaan verestä eristetyt valmisteet (autologous conditioned serum ja platelet-rich plasma) sekä botuliinitoksiini-A. Osa näistä vaihtoehdoista on jo kliinisessä käytössä.

Minkään kirjallisuuskatsauksessa käsitellyn lääkeaineen tehosta ei ole vankkaa tieteellistä näyttöä koiran nivelrikon hoidossa. Koska nivelinjektioihin liittyvät riskit ovat kuitenkin yleisesti varsin pienet, voi nivelensisäinen lääkitys kuitenkin olla joissain tapauksissa kokeilun arvoista. Kirjallisuuskatsausta voidaankin käyttää apuna suunniteltaessa koiralle nivelensisäistä kortikosteroidi- tai hyaluronihappolääkitystä.

Avainsanat – Nyckelord – Keywords

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OVERVIEW OF CANINE OSTEOARTHRITIS

1. ANATOMY OF THE SYNOVIAL JOINT

Joints can be divided into the three main categories of synovial, cartilaginous and fibrous joints depending on their degree of mobility (Piermattei et al. 2006; Liebich et al. 2007; Sjastaad et al. 2007). The clinically important joint diseases, such as osteoarthritis, affect mainly the synovial joints that have the highest range of motion (Piermattei et al. 2006). Examples of the synovial joints are the hip and elbow joints (Sjastaad et al. 2007). Fibrous joints can be found for example between the bones of the skull in young animals, while the joints between the vertebrae are examples of the cartilaginous joints (Sjastaad et al. 2007).

All synovial joints, also known as the true joints, consist of a joint capsule, articular cartilage, subchondral bone and synovial fluid (Piermattei et al. 2006; Sjastaad et al. 2007;Liebich et al. 2007).

1.1. Joint capsule

The two layers of the joint capsule are the outer fibrous layer and the inner layer also known as the synovial membrane (Liebich et al. 2007). The nerves, blood vessels and lymphatic vessels are located between the fibrous capsule and the synovial membrane (Schulz 2007).

The inner surface of the joint capsule is lined by the synovium that contains the synoviocytes (Edwards 2011). There are two types of synoviocytes (Liebich et al. 2007). Type A synoviocytes resemble macrophages by clearing debris by phagocytosis while the type B synoviocytes resemble fibroblasts and produce hyaluronic acid, proteins and potentially degenerative enzymes (Liebich et al. 2007; Edwards 2011).

1.2. Synovial fluid

The joint cavity is filled with synovial fluid that is formed as a dialysate of plasma from the blood vessels of the synovial membrane (Schulz 2007). Synovial fluid contains hyaluronic acid, sugar, electrolytes and enzymes that are vital for the nutrition of the

cartilage (Liebich et al. 2007). The hyaluronic acid in the synovial fluid lubricates the joint, acts as a shock absorber and decreases the friction caused by the joint movement (Venable 2008).

1.3. Articular cartilage and subchondral bone

The articular cartilage is a 1 to 5 mm thick layer of hyaline cartilage that facilitates the gliding movement of the joint, distributes mechanical loads and protects the underlying subchondral bone from injury (Schulz 2007; Sjastaad et al. 2007; Mobasheri 2010). The mechanically resilient extracellular matrix of hyaline cartilage consists mainly of the cartilage-specific type II collagen and aggregating proteoclycans (Mobasheri 2010; Garvican 2010). Proteoglycans consist of a protein core with covalently attached glycosaminoglycan side-chains (Bliss et al. 2012). Examples of glycosaminoglycans are keratin sulfate and chondroitin sulfate (Bliss et al. 2012).

Articular cartilage is joined with cancellous bone by subchondral bone which is a thin layer next to the epiphysis (Liebich et al. 2007). Subchondral bone acts as a shock-absorber as its interdigitated junction with cartilage helps to transform shear forces into tensile and compressive forces (Garstang et al. 2006). Subchondral bone also contains end arteries and veins that serve in the nutrition and waste product removal of articular cartilage (Garstang et al. 2006).

Despite its durability, the self-maintaining capacity and ability to response to injury of cartilage are very limited as it is metabolically quite inactive and lacks blood supply (Sjastaad et al. 2007; Schindler et al. 2011; Mobasheri et al. 2010). Articular cartilage also lacks nerve and lymph supply (Mobasheri et al. 2010; Schindler et al. 2011). Cartilage lesions that do not reach the subchondral bone do not usually repair spontaneously and the repair of full thickness lesions depends on the factors such as the size and location of the lesion (Koga et al. 2009). While small defects can be repaired with the production of hyaline cartilage, larger defects can only be repaired with the production of fibrous tissue or fibrocartilage that lacks the biochemical and biomechanical properties of hyaluronic cartilage (Koga et al. 2009).

The only cell type in cartilage, the chondrocyte, is solely responsible for the synthesis and turnover of the cartilage extracellular matrix (Mobasheri et al. 2010). Chondrocytes secrete macromolecular components such as collagen,

glycosaminoglycans and hyaluronic acid and modulate the extracellular matrix turnover and by this way maintain homeostatic synthesis and degradation of the extracellular matrix (Mortellaro 2003; Black et al 2008). The secretion of lytic and tissue-damaging mediators such as cytokines, free radicals, proteases and prostaglandins is controlled by anabolic and reparative substances such as growth factors and inhibitors of catabolic cytokines and inhibitors of degradative enzymes (Mortellaro 2003; Black et al 2008).

The major part of the nutrients reaches the articular cartilage by diffusion from the synovial fluid (Liebich et al. 2007; Garvican 2010). Some transport of nutrients to articular cartilage can also occur from the joint synovia or from the blood vessels of the bone marrow (Liebich et al. 2007; Garvican 2010).

1.4. Additional structures

The surrounding tendons provide external support for the joint (Schulz 2007). Joints also contain intracapsular, capsular or extracapsular ligaments that add to the stability of the joint (Liebich et al. 2007). In addition to these, in some joints there are also fibrocartilagenous structures, such as the menisci in the knee joint, that further help to stabilize the joint (Liebich et al. 2007). Also other structures facilitating the joint function, such as fat pads, can be found in certain joints (Schulz 2007).

2. ARTHROPATHIES

2.1. Classification of arthropathies

Diseases affecting the joints, also known as arthropathies, can be divided into the two major categories of inflammatory and noninflammatory arthropathies based on the disease etiology (Schulz 2007). Inflammatory arthropathies can be further divided into the groups of immune-mediated, infective and crystal-induced disease processes (Innes 2012). The most common joint disease in dogs in the category of inflammatory arthropathies is immune-mediated nonerosive polyarthritis (IMPA) that is characterized by immune-complex deposition in the synovium resulting in synovitis and inflammation in the joint (Taylor 2009).

Several developmental, degenerative, neoplastic and traumatic joint disorders fall in the category of non-inflammatory joint disorders (Taylor 2009). Some of the diseases in this category are osteoarthritis, coagulopathic arthritis and traumatic arthritis (Innes 2012). Of these, osteoarthritis is the most common one, as it is the most common of all joint diseases in dogs (Taylor 2009; Innes 2012).

2.2. Diagnostic approach to joint disease

The anamnesis of an animal with a joint disease usually includes a history of lameness or abnormal gait (Taylor 2009). Inflammatory arthropathies might also cause signs of systemic illness such as fever and depression (Taylor 2009). Polyarthritis, meaning the concurrent inflammation of several joints, may not always cause obvious lameness but instead might lead to less specific signs such as decreased appetite, fever, weakness, stiffness and exercise intolerance (Schulz 2007).

A thorough physical examination should always be performed even if the anamnesis clearly points to an orthopedic problem. Physical examination might reveal other health problems that might be related to an orthopedic disease or influence the patient's suitability for sedation or a certain treatment option. (Arthurs 2011.)

Orthopedic examination should be performed on all patients with a history of lameness, exercise intolerance, collapses or ataxia or that are recumbent (Arthurs 2011). Abnormalities attributable to an arthropathy, in addition to lameness or abnormal gait, include changes in the range of motion, pain, instability and crepitation on the manipulation of the affected joints (Schuz 2007). Joint enlargement and heat might also be found along with asymmetrical musculature due to shifting of the weight to the unaffected side (Schulz 2007).

After localizing the problem to a joint by physical and orthopedic examination, further diagnostic tools include collecting the minimum database (complete blood count, serum biochemistry and urinalysis), radiography possibly accompanied by other imaging modalities such as computed tomography and magnetic resonance imaging and possibly a synovial fluid analysis and culture (Taylor 2009). Synovial membrane biopsy and immunologic and serologic testing might also be appropriate in certain cases (Taylor 2009).

There should be no abnormal changes in the complete blood count (CBC), serum biochemistry and urinalysis in cases of non-inflammatory joint diseases, but for example certain infectious arthropathies may cause abnormal findings such as leukocytosis (Taylor 2009).

Radiographs should be taken in cases where there are abnormal findings on joint manipulation, such as pain, swelling, crepitation, instability or restricted range of motion (Taylor 2009). Signs of a joint disease seen on radiographs include the increased volume of synovial fluid, changes in the width of the joint space, changes in the opacity of subchondral or perichondral bone, subchondral cyst formation, perichondral bone proliferation, also known as osteophyte formation, mineralization of the articular soft tissues or intra-articular mineralized fragments and abnormalities in the joint congruity (Allan 2007). Abnormal findings on radiographs can be expected especially in cases of osteoarthritis, chronic septic arthritis and immune-mediated erosive arthritis (Taylor 2009). However, one should note that the radiographic abnormalities are usually non-specific and might not be seen until weeks to months after the onset of clinical signs (Taylor 2009).

When suspecting a joint disease, the most useful diagnostic test is synovial fluid analysis that should be performed when signs of joint disease are found during the previous diagnostic steps (Clements 2006; Taylor 2009). Synovial fluid analysis should also be performed in cases where polyarthritis or sepsis is suspected or when there are potential signs of immune-mediated joint disease such as fever of unknown origin (Clements 2006; Taylor 2009).

Synovial fluid analysis helps not only to confirm or rule out joint disease as a cause of lameness or other clinical signs, but it also helps to evaluate the treatment response in cases of previously diagnosed inflammatory joint diseases (Clements 2006). It is also useful in differentiating between different types of arthropathies as shown on the Table 1 (Schulz 2007; Taylor 2009).

The volume, colour, viscosity and cloudiness of synovial fluid are also evaluated. The normal synovial fluid is colorless or light yellow, viscous and clear (Clements 2006). Laboratory analysis provides information on the cytology and relative numbers of the different cell types (Clements 2006).

Table 1. Typical total and differential cell counts for canine synovial fluid in normal joints and in different joint diseases (Innes 2012).

Condition	Total cell count	Percentage of mononuclear cells	Percentage of neutrophils
Normal	$<2 \times 10^9$ /L	94-100	0-6
Osteoarthritis	$2\text{-}5 \times 10^9$ /L	88-100	0-12
Rheumatoid arthritis	$8\text{-}38 \times 10^9$ /L	20-80	20-80
Nonerosive IMPA	$4\text{-}370 \times 10^9$ /L	5-85	15-95
Infective arthritis	$40\text{-}267 \times 10^9$ /L	1-10	90-100

3. OSTEOARTHRITIS

3.1. Overview

Osteoarthritis (OA, also known as degenerative joint disease, hypertrophic arthritis, degenerative arthritis and osteoarthrosis) can be defined as a process of abnormal repair and progressive loss of articular cartilage (Schulz 2007; Garvican 2010). Osteoarthritis affects all mammals and it is an important and costly disease in dogs, horses and humans (Venable 2008; Garvican 2010). It is a common cause of chronic pain and the most common type of arthritis in dogs as it has been estimated that about 20 % of adult dogs suffer from it (Allan 2007; Innes 2012). Osteoarthritis is the most important chronic musculoskeletal disease in horses and a significant cause of economic losses in the equine industry (Van Wereen 2010). It is the most common arthropathy also in humans as it affects millions of people in the western countries (Mele 2007; Chevalier 2010).

The pathophysiological and clinical features of osteoarthritis as well as the treatment responses and the anatomy of stifle joint are very similar in dogs compared to humans. Thus the dog is the most commonly used animal model for the studies on osteoarthritis. (Cook 2010.)

3.2. Etiology

Osteoarthritis can be divided into the primary or secondary forms based on whether or not an underlying cause can be identified (Schulz 2007). In primary, or idiopathic, osteoarthritis, which is more common in humans than in dogs, there is no known cause for the disease development (Schulz 2007; Innes 2012). Some of the factors that can lead to the development of secondary osteoarthritis are alterations in the joint biomechanics, such as joint instability and abnormal loading of the articular cartilage or disorders leading to the formation of abnormal cartilage (Schulz 2007; Innes 2012). Common causes of secondary osteoarthritis in dogs are hip dysplasia and cranial cruciate ligament rupture (Schulz 2007).

The risk factors of osteoarthritis can be divided into two major categories: systemic and local risk factors (Garstang 2006). Local factors such as prior joint trauma, overload, instability, muscle weakness and developmental abnormalities alter the biomechanical loading of affected joints (Garstang 2006; Schulz 2007). The role of systemic factors in the disease development is more obscure and less studied in dogs compared to humans (Innes 2012). Examples of systemic risk factors are genetic factors, high age, nutritional factors, gender and hormonal status (Garstang 2006; Innes 2012). In dogs no genes predisposing to the development of osteoarthritis have yet been identified (Innes 2012).

Obesity is an important risk factor that is likely to have both a systemic and local component (Garstang 2006; Innes 2012). Increased load on the joint coupled with altered joint alignment was for a long time thought to be the only mechanism by which obesity predisposes to the development of osteoarthritis (Innes 2012; Sanderson 2012). However, based on recent research the adipokines secreted by adipose tissue, such as tumor necrosis factor (TNF), interleukin-6 (IL-6) and leptin, are also likely to play a role in the pathogenesis of osteoarthritis by generating systemic low-level pro-inflammatory conditions that affect the joint metabolism (Innes 2012; Sanderson 2012). Indeed, maintaining optimal body condition has been shown to decrease the progression of osteoarthritis in dogs (Innes 2012).

3.3. Pathogenesis

For a long time osteoarthritis was seen as a result of simple wear and tear, until about three decades ago, when the role of cellular mechanisms in the pathogenesis of osteoarthritis was recognized (Evans et al. 2005). Despite this discovery and the vigorous research the pathophysiology of osteoarthritis is still not fully understood but it is likely to involve a combination of mechanical, biological, biochemical, molecular and enzymatic processes (Chevalier & Kemta-Lepka 2010).

Although the hallmark of the disease process is abnormal repair and gradual degradation of articular cartilage, osteoarthritis causes changes also in all of the other structures of the synovial joint including the synovial membrane, synovial fluid and subchondral bone (Mateescu et al. 2008; Garvican et al. 2010; Innes 2012). Osteophyte formation is also typical in osteoarthritis and it is considered as an attempt to limit both movement and pain that occur in response to the chronic inflammation and local tissue damage (Mele et al 2007; Schulz 2007; Innes 2012). In addition to articular structures, osteoarthritis also affects other tissues as the decreased use of the affected limb weakens the surrounding muscles, ligaments and tendons (Garstang et al. 2006; Innes 2012).

In osteoarthritis there is a relative overproduction of catabolic and pro-inflammatory mediators to their inhibitors leading to a catabolic state in the articular cartilage and eventually to its progressive destruction (Mortellaro 2003; Black et al. 2008). The release of degradation products from the extracellular matrix of articular cartilage due to mechanical or enzymatic destruction can cause the release of catabolic and pro-inflammatory mediators such as cytokines IL-1, IL-6 and TNF-α, nitric oxide and destructive enzymes by chondrocytes and synovial cells (Chevalier & Kemta-Lepka 2010; Mobasheri & Henrotin 2010; Innes 2012). This initiates an inflammatory response that alters the normal balance of cartilage matrix degradation and repair (Chevalier & Kemta-Lepka 2010; Mobasheri & Henrotin 2010). The decreased synthesis of the inhibitors of the aforementioned pro-inflammatory mediators causes further damage to the articular cartilage (Schulz 2007; Innes et al. 2010a; Innes 2012). Diseased cartilage is more susceptible to mechanical stress and further damage and thus the vicious cycle of inflammation and cartilage destruction is initiated (Schulz 2007). Grossly the deterioration of articular cartilage is initially seen as fibrillation of

the superficial layer of the joint cartilage that ultimately proceeds to deeper fissures eventually reaching the subchondral bone (Schulz 2007; Innes 2012).

The importance of the cellular mechanisms and inflammatory mediators in the pathogenesis of osteoarthritis has been pointed out in studies such as the one by Xu et al. (2009) that investigated the effect of biological factors in the synovial fluid of an osteoarthritic joint on normal articular cartilage. Osteoarthritis of the stifle joint was induced surgically and confirmed 24 weeks later in eight dogs. After this, an elbow joint of each dog was injected with synovial fluid from the osteoarthritis stifle joint of the same dog while the other elbow was injected with the same volume of saline. The injections were performed once a week for 24 weeks. Significant morphological changes in the articular cartilage of the elbow joint were observed 48 after the surgery. These changes included degeneration of chondrocytes and cartilage matrix along with changes in the superficial layer of the cartilage evaluated by electron microscope. Almost no differences in the articular cartilage between the saline injected joints and in the joints of the control group were noted.

Despite being classified as a non-inflammatory arthropathy, osteoarthritis involves synovial inflammation, the degree of which varies across different synovial sites and over time (Schulz 2007; Chevalier & Kemta-Lepka 2010; Innes 2012). Synovitis is often associated with increased capillary permeability and subsequent leakage of serum proteins which leads to synovial edema and increased synovial fluid volume (Edwards 2011).

3.4. Diagnosis

3.4.1. Anamnesis

The clinical signs of osteoarthritis are similar to those of other joint disease (Innes 2012). The most common of these is lameness, but other complaints such as stiffness after rest and reluctance to exercise or jump are also common (Schulz 2007; Innes 2012). Behavioral changes caused by pain are also possible and can be either overt or very subtle and thus easily missed (Hellyer et al. 2007; Innes 2012). The clinical signs can worsen over time or they can be intermittent with periods of disease flares (Innes

2012). They can also be affected by external factors such as changes in exercise or weather (Innes 2012).

Osteoarthritis can affect any joint and dogs of all sizes and breeds can be affected as they age (Rychel 2010). The dog might have a history of having a predisposing condition such as another type of arthritis, previous joint trauma, joint or patellar luxation, hip or elbow dysplasia or cranial cruciate ligament rupture (Schulz 2007).

3.4.2. Minimum database

As stated earlier, there are no abnormal findings in the complete blood count, serum biochemistry or urinalysis caused by osteoarthritis (Taylor 2009). However, collecting minimum database is useful for identifying possible concurrent diseases that might limit the therapy options.

3.4.3. Clinical examination

Lameness is a common finding in the clinical examination, unless the dog suffers from a bilateral condition in which case more subtle signs such as a continuous shifting of the weight while standing or shortened stride may be seen (Schulz 2007). Other signs such as muscle atrophy and abnormal changes in the palpation and manipulation of the affected joint such as joint swelling or effusion, capsular or extracapsular fibrosis, diminished range of motion, crepitus and pain can also be found (Innes 2012).

A predisposing condition such as a rupture of the cranial cruciate ligament or joint incongruity caused by trauma or angular limb deformity might also be identified during the clinical examination (Taylor 2009). Systemic signs such as fever and depression that are common in inflammatory joint diseases do not accompany osteoarthritis (Taylor 2009).

3.4.4. Radiography

The radiographic changes of osteoarthritis vary depending on the stage of the disease process (Allan 2007). In the early stages of osteoarthritis there are typically no

radiographic findings but as the disease progresses radiographic changes become evident (Allan 2007).

The most readily recognizable radiographic change is osteophyte formation despite the fact that it is not pathognomonic for osteoarthritis (Allan 2007; Innes 2012). The osteophytes are formed in the articular margins and attachment sites of the joint capsule, tendons and ligaments (Marino & Loughin 2010). Osteophytes at the sites where a tendon or ligament attaches to bone are also known as enthesophytes (Allan 2007). In the stifle joint osteophytes are most commonly found in the trochlear ridges, patella, fabella, attachment sites of the ligaments and the caudal tibial plateau (Marino & Loughin 2010). In the coxofemoral joint osteophytes are most commonly seen on the cranial or caudal margins of the acetabulum and also in the femoral head and neck (Kapatkin et al. 2002).

Other common radiographic signs of osteoarthritis are the synovial fluid effusion and increased opacity, also known as sclerosis, of the subchondral bone (Allan 2007). In the stifle joint synovial effusion causes proximal displacement of the infrapatellar fat pad and caudal displacement of the joint capsule (Marino & Loughin 2010).

Changes in the width of the joint space are also possible as the joint space may initially appear widened, but later thinner than normal due to the loss of cartilage. (Allan 2007) However, to assess the joint space width, the radiographs should be taken when the animal is bearing weight on the limb which is rarely done with dogs (Marino & Loughin 2010; Innes 2012). Soft tissue mineralization of the joint structures may also be noted on radiographs (Allan 2007).

Radiography has certain limitations that need to be appreciated. As the articular cartilage is not visible on radiographs, radiography provides information mostly on the bony changes which might not be evident at the onset of clinical signs (Allan 2007; Innes 2012). One should also note that although radiographs are useful in diagnosing osteoarthritis, the radiographic findings might not correlate well with limb function and thus their use in the evaluation of the disease progression has been questioned (Gordon et al. 2003). Despite the radiographic evidence of osteoarthritis, many animals are asymptomatic and might not develop lameness as they age (Schulz 2007).

3.4.5. Synovial fluid analysis

As mentioned above, synovial fluid analysis should be performed in all cases of suspected joint disease and it helps in distinguishing osteoarthritis from inflammatory arthropathies (Innes 2012). In the case of osteoarthritis the inflammatory changes on cytology of the synovial fluid are typically mild, but there can be gross changes such as the decrease in the viscosity and increase in the volume of synovial fluid (Taylor 2009; Innes 2012). Cellular changes attributable to osteoarthritis are summed up in Table 1.

3.4.6. Others

Some of the limitations of radiography can be overcome by using other imaging diagnostic tools. Of these arthroscopy is currently considered to be the most sensitive as it allows evaluation of the degree of cartilage damage and synovial proliferation in addition to assessment of other intra-articular structures at earlier stages than radiography (Schulz 2007; Innes 2012). Other imaging options in osteoarthritis that can also be considered are magnetic resonance imaging (MRI) and computed tomography (CT), the former of which is generally more useful, as it provides information also on the soft tissue structures of the synovial joint (Innes 2012). However, as both MRI and CT are expensive studies to perform, they are used practically in the diagnosing of osteoarthritis only in human medicine.

The early stages of osteoarthritis are difficult to diagnose as alterations in joint structure and function occur before the clinical signs of osteoarthritis, which prevents early diagnosis and thus early initiation of treatment that could potentially help to delay the disease process (Matyas et al 2004; Venable et al 2008). There is also no effective way to monitor the disease progression (Mobasheri & Henrotin 2010). Thus other diagnostic tools that might fill the areas of weakness of the other diagnostic procedures are being developed. An example of this is the use of cartilage breakdown-products as biomarkers for osteoarthritis (Garvican et al. 2010; Garvican et al. 2012). An ideal biomarker would bring information on the cartilage homeostasis, could be measured from a routine blood or urine sample before the onset of clinical signs and thus help in starting the treatment to prevent the development of osteoarthritis (Venable et al. 2008; Mobasheri & Henrotin 2010; Garvican et al. 2012). However,

finding such a biomarker can be challenging, mostly due to the fact that as the greatest proportion of cartilage in the body is found in the spine and respiratory system, the release of breakdown products from a single diseased joint may be relatively small (Garvican et al. 2010; Garvican et al. 2012).

MANAGEMENT OF CANINE OSTEOARTHRITIS

1. OVERVIEW

There is currently no known cure for osteoarthritis as there are no approved structure- or disease-modifying therapies that could protect the articular cartilage from further damage in osteoarthritis or affect the pathways of disease progression (Mobasheri & Henrotin 2010; Kwon & Park 2012). This, together with the fact that the diagnosis of osteoarthritis is often made in the late and irreversible stages, means that the treatment is mainly palliative as the medications only provide variable symptomatic relief from pain and inflammation (Johnston et al. 2008; Venable et al. 2008; Mobasheri & Henrotin 2010; Spakova et al. 2012). Even in human medicine there is currently very little that can be done for patients with mild-to-moderate osteoarthritis (Evans 2005). Thus there is a continuous search for better treatment options and studies are focused on the treatment options such as cytokine inhibitors, gene therapy and applications of growth factors that might have potential to preserve normal joint homeostasis or even reverse the structural damage in degenerative joints (Johnston et al. 2008; Filardo et al. 2012; Spakova et al. 2012). Such a drug capable of decreasing the disease progression is already available for the treatment of rheumatoid arthritis in humans (Chevalier & Kemta-Lepka 2010).

Of the treatment goals for osteoarthritis, the alleviation of pain is the most important (Mobasheri & Henrotin 2010). Pain management allows the patient to gain strength and maintain mobility (Mobasheri & Henrotin 2010; Rychel 2010). Other treatment goals include the maintenance of joint mobility, improvement of the patient's quality of life and potentially slowing down the disease process (Kuroki et al. 2002; Gigante & Callegari 2011).

The treatment of osteoarthritis is most often described as multimodal (Johnston et al. 2008). This means that it relies on a combination of different types of therapies such as administration of NSAIDs and other analgesics, nutraceuticals and functional foods, physical therapy and so-called alternative therapies such as acupuncture (Aragon et al. 2007; Sanderson et al. 2009).

Often the treatment decisions are affected by various different factors such as the practitioner's personal experience, the compliance and goals of the owner and the

resources available (Johnston et al. 2008; Rychel 2010). Most canine patients are managed with conservative treatment options such as NSAIDs, nutritional supplementation, physiotherapy and weight management (Innes 2012). One should note that as clinical signs do not always correlate with the radiographic signs of osteoarthritis, it is important to tailor the treatment of osteoarthritis based on the patient's symptoms instead of radiographic findings (Schulz 2007).

2. TREATMENT OPTIONS

2.1. Pharmacological management

Based on the systematic review of clinical studies on the treatment options for osteoarthritis by Aragon et al. (2007), meloxicam has the strongest proof of efficacy in the management of canine osteoarthritis. Therapy options with a moderate proof of efficacy included carprofen, etodolac, pentosan polysulphate and polysulphated glycosaminoglycans.

Meloxicam along with carprofen and firocoxib was evaluated to have the strongest evidence of clinical effect for the treatment of osteoarthritis also in the systematic review by Sanderson et al (2009). Glycosaminoglycan polysulphate was evaluated to have moderate evidence of effectiveness while the strength of evidence for the use of pentosan polysulphate was evaluated to be weak.

2.1.1. Analgesics

2.1.1.1 Non-steroidal anti-inflammatory drugs

The non-steroidal anti-inflammatory drugs are the most commonly used class of medication in the management of osteoarthritis (Johnston et al. 2008). This is due to their scientifically proven effect in the palliation of acute and chronic pain that starts relatively shortly after administration and last for a considerably long time (Lamont & Mathews 2007; Johnston et al. 2008; Innes et al. 2010a). Their relative ease of administration also contributes to their popularity (Johnston et al. 2008).

Some practitioners prefer to use NSAIDs only as needed while others recommend their continuous use (Innes et al. 2010b). The continuous use of NSAIDs has been associated with better control of pain and better use of the joint leading to improved mobility (Innes et al. 2010b). Also continuous nitric oxide inhibition by NSAIDs in the articular cartilage might at least theoretically reduce the cell death and thus potentially retard the progression of osteoarthritis (Innes et al. 2010b). The amount of long-term studies on the adverse effects of NSAIDs is scarce (Lamont & Mathews 2007). However, in the systematic review by Innes et al. (2010b), the risk of serious adverse effects of long-term use of NSAIDs was reported to be low based on the currently available data.

Even if NSAIDs are not used on a daily basis, they are beneficial during the acute flares of inflammation that are common in osteoarthritis. In these cases a short course of NSAIDs together with the rest of 2 to 3 days may be needed. When the phase of acute inflammation has subsided, normal activity level can be gradually restored. (Schulz 2007.)

Carprofen and meloxicam are examples of the NSAIDs commonly used in the management of canine osteoarthritis (Innes 2012). Carprofen can be given as a dose of 4,4 mg/kg once a day or 2,2 mg/kg twice a day (Plumb 2011). In the long-term use the dose is advised to be lowered down to 2 mg/kg given once a day (Lääketietokeskus 2013). Meloxicam is advised to be given at a dose of 0,2 mg/kg on the first day of treatment and at a dose of 0,1 mg/kg once a day on subsequent days (Plumb 2011).

- Mechanism of action

The action of NSAIDs in mediated via the inhibition of the enzyme cyclooxygenase (COX) that oxidizes arachidonic acid to eicosanoids such as prostaglandins (PGs) (Lamont & Mathews 2007; KuKanich et al. 2012). The two main forms of COX are COX-1 and COX-2 (Lamont & Mathews 2007; KuKanich et al. 2012). The prostaglandins produced by these enzymes have been shown not only to have important physiological functions throughout the body, but to be up-regulated upon various stimuli during illness (Lamont & Mathews 2007; KuKanich et al. 2012).

Some of the prostaglandins, such as prostaglandin E_2 (PGE_2), produced by COX-1 are vital for the mucosal defense of the gastrointestinal tract (Lamont & Mathews 2007;

KuKanich et al. 2012). PGE_2 increases the mucus production and turnover of mucosal cells in the gastrointestinal tract, decreases secretion of gastric acid and increases bicarbonate secretion in the duodenum (KuKanich et al. 2012). Another eicosanoid produced by COX-1, thromboxane A_2 (TXA_2), is necessary for the function of platelets and enhances blood clot formation and coagulation (Lamont & Mathews 2007; KuKanich et al. 2012). Thus the inhibition of COX-1 has an anticoagulant effect (KuKanich 2012).

Also the prostaglandins produced by COX-2 have important functions in the body. For example, they take part in the prevention and healing of mucosal injuries, inhibit the adherence of leukocytes and have important physiological functions in the kidneys (Lamont & Mathews 2007). Especially PGE_2 and prostacyclin (PGI_2) are important for the normal function of the kidneys as they increase the excretion and inhibit the reabsorption of sodium and stimulate the release of renin (KuKanich et al. 2012). COX-2 seems to have also other important physiologic functions in the body such as those associated with the nervous and reproductive systems and bone metabolism (Lamont & Mathews 2007).

Prostaglandins, especially PGE_2 and PGI_2, are also mediators of pain and inflammation and thus their inhibition in osteoarthritis is desired (Lamont & Mathews 2007). NSAIDs seem also to have central antinociceptive effects at the spinal and supraspinal levels (Lamont & Mathews 2007; Innes 2012). There is also evidence that NSAIDs might have a direct effect on cellular mechanisms of osteoarthritis at the joint level, potentially through the inhibition of cell death in articular cartilage induced by nitric oxide (Innes et al. 2010b).

Different NSAIDs vary in regards to which form of COX they inhibit (Lamont & Mathews 2007). Some of the NSAIDs that inhibit preferentially COX-2 are meloxicam and carprofen while ketoprofen and aspirin are examples the NSAIDs that inhibit both COX-1 and COX-2 (Lamont & Mathews 2007).

- Potential side effects and contraindications

The main adverse effects of NSAIDs are associated with the gastrointestinal tract, the kidneys and the impairment of platelet activity (Innes 2012). As dogs are more susceptible to the side effects of NSAIDs than people and most of the drugs in this

class have narrow safe margins, accurate dosing is vital (Lamont & Mathews 2007). Due to individual variations in response to different NSAIDs, switching NSAIDs to determine which one is the most effective for the patient may be advisable, especially if controlling pain requires high doses of a particular NSAID (Lamont & Mathews 2007; Taylor 2009). In these cases a washout period of minimum of 3 days should be left before starting the new drug to reduce the risk of adverse effect (Taylor 2009). Concurrent use of NSAIDs with glucocorticoids should also be avoided as it has been linked with increased risk of side effects, especially gastrointestinal ulceration (KuKanich et al. 2012).

Most of the adverse effects caused by NSAIDs are considered to be associated with the gastrointestinal tract (KuKanich et al. 2012). As weak acids NSAIDs can cause direct irritation of the mucosa after oral administration or following secretion in bile after hepatic elimination (KuKanich et al. 2012). However, the adverse effects are also mediated indirectly through the inhibition of PGE_2 and PGI_2 since these eicosanoids help to protect the gastrointestinal mucosa from injury as stated earlier (Innes et al. 2010a; KuKanich et al. 2012). Other potential mechanisms by which NSAIDs may exert adverse effects on the gastrointestinal tract are increased production of leukotrienes and inhibition of aspirin triggered lipoxin (KuKanich et al. 2012). Thus NSAIDs should be avoided or at least used cautiously in patients with gastrointestinal damage due to ulceration or intestinal surgery (KuKanich et al. 2012).

Other contraindications of NSAIDs are acute renal failure, dehydration and coagulopathies (Lamont & Mathews 2007). Due to the inhibition of PG activity, NSAIDs may also be harmful for reproductive function and should not be used during pregnancy (Lamont & Mathews 2007). Since most NSAIDs are eliminated by the liver, hepatic disease might be a relative contraindication for the use of NSAIDs, as it can lead to increased drug exposure and thus increase the risk of adverse effects (KuKanich et al. 2012). Using NSAIDs in animals with disorders causing impaired visceral perfusion such as heart failure can increase the risk of gastrointestinal ulceration (Taylor 2009). A thorough physical examination and collecting minimum database including complete blood count, serum biochemistry and urinalysis should be performed prior to starting a NSAID therapy to help to rule out possible contraindications for NSAID use (Rychel 2010).

The symptoms to be monitored during NSAID therapy include hematochezia or melena, vomiting, increased water consumption and nonspecific changes in demeanor (Lamont & Mathews 2007). In the long-term use creatinine and alanine aminotransferase monitoring is recommended (Lamont & Mathews 2007).

Clinical signs that may be caused by gastric ulceration include depression, reduced appetite, vomiting, diarrhea and digested blood in the vomitus or feces, although some dogs with gastric ulceration don't show any obvious symptoms (KuKanich et al. 2012). If such signs occur during the therapy, the administration of NSAIDs should be discontinued until the signs subside (KuKanich et al 2012). After the clinical signs have resolved, several approaches can be taken, although their safety or efficacy has not been evaluated (KuKanich et al. 2012). The administration of NSAIDs can be continued with a concurrent administration of a gastroprotectant such as omeprazole, famotide or misoprostol (KuKanich et al. 2012). Other options are reducing the drug dose by adding an analgesic drug of another class or switching to another type of analgesic or to a different NSAID (Lamont & Mathews 2007; KuKanich et al. 2012).

2.1.1.2. Other analgesic drugs

Combining other analgesic drugs with NSAIDs has certain benefits in treating chronic pain (Lamont & Mathews 2007). Not only can they help to control pain refractory to NSAIDs, but they may also enable the dose reduction of NSAIDs thus reducing the risk of NSAID-induced side effects (Lamont & Mathews 2007). They might also be of benefit for animals with significant liver, renal or gastrointestinal disease or for patients that do not tolerate NSAID administration (Rychel 2010). Examples of the analgesic adjuvants that can be used in the management of osteoarthritis include tramadol, amantadine, gabapentine and amitriptyline (Lamont & Mathews 2007; Plumb 2011; KuKanich et al. 2012). Although the aforementioned analgesic drugs are in clinical use, the scientific evidence on their efficacy in the treatment of canine osteoarthritic pain is scarce. Only the efficacy of amantadine has been investigated in a controlled clinical trial in osteoarthritic dogs (Johnston et al. 2008).

Tramadol is an opiate-like agonist with μ-receptor activity (Plumb 2011). It also inhibits the reuptake of serotonin and norepinephrine (Rychel 2010; Plumb 2011). It is commonly administered for veterinary patients combined with a NSAID (Johnston et

al. 2008). For mild chronic pain in dogs it is commonly administered per orally at doses of 2-5 mg/kg 2-3 times a day together with another type of analgesic such as a NSAID (Plumb 2011). However, dosage adjustments might be needed in patients with hepatic or renal disease (Plumb 2011). Tramadol is generally well tolerated in dogs (Plumb 2011). Possible side effects include sedation, anxiety and problems associated to the gastrointestinal tract such as vomiting, constipation and diarrhea (Plumb 2011).

Amantadine inhibits the N-methyl-D-aspartate (NMDA) receptors that are found in the dorsal spinal horn and whose activation is associated with chronic pain (Johnston et al. 2008; Rychel 2010). In the management of osteoarthritis, amantadine is not likely to be effective when administered as the only analgesic, but together with a NSAID it might be beneficial (Johnston et al. 2008; Plumb 2011). In a study by Lascelles et al. (2008) on the effect of amantadine used in combination with meloxicam was found beneficial in alleviating pain in osteoarthritic dogs. No side-effects were reported. Amantadine can be used to manage pain caused by osteoarthritis at doses of 3-5 mg/kg given perorally once a day (Plumb 2011). However, as amantadine is eliminated via the kidneys, dosage adjustments might be needed when it is used in dogs with renal disease (Plumb 2011).

Gabapentin has been found to be beneficial in the treatment of neurogenic pain although the mechanism by which gabapentin exerts its analgesic action is not completely understood (Johnston et al. 2008; Plumb 2011). However, it is thought to decrease the release of excitatory neurotransmitters by binding to the voltage-gated calcium channels (Plumb 2011). In the management of osteoarthritis the doses of 5-10 mg/kg twice daily with or without together a NSAID can be used (Plumb 2011). As gabapentin is eliminated via the kidneys, dose adjustments might be needed in dogs with severe renal dysfunction (Plumb 2011).

The most common side effect of gabapentin is sedation. Thus it is recommended to be started with a lower dose. Sudden discontinuation of gabapentin should also be avoided, because it has been associated with potential withdrawal-precipitated seizures. (Plumb 2011.)

Amitriptyline is primarily used to treat behavioral conditions such as anxiety, but it has also potential in the management of neuropathic pain (Johnston et al. 2008; Plumb 2011). Amitriptyline is a tricyclic antidepressant drug that causes the blockage of the amine pump leading to increased neurotransmitter levels, sedation and

anticholienergic activity (Plumb 2011). It also is binds to the H_1 receptors, sodium channels and glutamate receptors (Plumb 2011). Doses of 1-2 mg/kg once or twice a day can be used in the management of canine osteoarthritis (Plumb 2011). As amitriptyline is metabolized in the liver, it should be used cautiously in patients with hepatic disease (Plumb 2011). The most common side effects are sedation and anticholinergic effects such as constipation and urinary retention (Plumb 2011).

2.1.2. Other pharmacological treatment options

Other pharmacological treatment options include polysulfated glycosaminoglycan and pentosan polysulfate (Innes 2012). Although they are used intra-articularly in the equine practice, there are currently no reports on their use in dogs (McIlwraith 2011). Polysulfated glycosaminoglycan and pentosan polysufate might have structure-modifying effects in the treatment of osteoarthritis, although this is yet to be proven (Innes 2012).

Pentosan polysulfate is a semisynthetic glycosaminoglycan that can be used in the management of canine osteoarthritis for its potential chondroprotective functions (Plumb 2011; Innes 2012). In Finland pentosan polysulfate is available only as an injectable solution (Carthrophen® 100 mg/ml) that is administered subcutaneously as a dose of 3 mg/kg as four injections with 5-7 days apart (Plumb 2011; Lääketietokeskus 2013).

In a review by Hannon et al. (2003) of the reported side effects of pentosan polysulphate product Carthrophen Vet® in the UK the most commonly reported adverse effects were general changes in the dog's demeanour, such as inappetance and lethargy, and vomiting. It should also be noted that as pentosan poysulfate has potential anticoagulant effects, its concurrent use with a NSAID is not recommended (Plumb 2011).

The exact mechanism of action of polysulfated glycosaminoglycan (PSGAG) is not known, although it has been licensed for the treatment of canine osteoarthritis in some countries (Innes 2012). There is currently no licensed injectable drug formulation of polysulfated glycosaminoglycan for dogs in Finland. The proof of efficacy of PSGAG has also been evaluated to be only weak to moderate in two systematic reviews (Aragon et al. 2007; Sanderson et al. 2009). Polysulphated glycosaminoglycan can be

given as a dose of 4,4 mg/kg intramuscularly twice a week for up to 4 weeks (Plumb 2011). As PSGAG is a heparin analogue, its use in patients with a bleeding disorder and concurrent use with a NSAID should be avoided (Johnston et al. 2008).

2.2. Non-pharmacological management

2.2.1. Weight management

As discussed previously, obesity is a potential predisposing factor for the development of osteoarthritis both by increasing the risk of mechanical injury to the articular structures and by creating a state of chronic systemic inflammation (Rychel 2010; Innes 2012). Obesity may also be the result of long-term chronic pain that has led to the reluctance to exercise (Schulz 2007). Overweight pets with osteoarthritis are also more likely to be inactive, as extra weight increases the load on sore joints, and gain even more weight which initiates a vicious cycle (Rychel 2010). Since in addition to osteoarthritis, a higher incidence of other diseases such as diabetes mellitus, hepatic lipidosis, cardiovascular and respiratory problems and dermatitis has been linked to obesity, weight loss is advisable (Nelson et al. 2007).

Most common reasons for obesity are excessive caloric intake and reduced daily activity, although a genetic predisposition has been identified in certain breeds such as the Labrador Retriever and Cocker Spaniel. Obesity is less frequently caused by an endocrine disease such as hypothyroidism, hyperadrenocorticism or hyperinsulinism or drugs such as progestagens or corticosteroids. (Taylor 2009.)

There are studies that suggest that weight loss alone can ameliorate the clinical signs and improve mobility in obese dogs with osteoarthritis (Johnston et al. 2008). One of these studies was performed by Marshall et al (2010) on the effect of weight loss on lameness in fourteen obese dogs with osteoarthritis. An average of 8,6 % reduction of the initial body weight during the 16-week-long study period resulted in improvement in lameness assessed both visually and by kinetic analysis.

The rate of weight loss should be 1 to 2 % per week at maximum as a faster rate is more likely to lead to reduced patient and client compliance and loss of muscle mass instead of fat (Taylor 2009). This is usually achieved by reducing the caloric intake to 80 % and then adjusting the diet based on regular weigh-ins (Taylor 2009). Regular

veterinary check-ups are an important part of the weight loss program (Schulz 2007; Taylor 2009).

2.2.2. Physical rehabilitation and exercise adjustments

Physical rehabilitation in the management of osteoarthritis includes the use of exercise, massage, heat, cold, water, sound and electricity to improve function and reduce pain (Dunning & Lascelles 2007; Johnston et al. 2008; Rychel 2010). Increased circulation and lymph flow in the affected area, the prevention of muscle atrophy and reduction of inflammation are examples of the potential beneficial effects of physical rehabilitation (Johnston et al. 2008).

Local hypothermia, also known as cryotherapy, means the application of therapeutic cold on musculoskeletal tissues. It is most useful in acute cases of inflammation, such as less than 72 hours after an injury or during the acute exacerbations of osteoarthritis (Dunning & Lascelles 2007; Rychel 2010). Local hypothermia minimizes edema through vasoconstriction, decreases enzyme activity and metabolism in tissues and provides analgesia by affecting the nerve conduction of the sensory nerves and by causing relaxation of the skeletal muscle (Dunning & Lascelles 2007). Cryotherapy should be used for 5 to 15 minutes at a time up to four times a day (Dunning & Lascelles 2007; Rychel 2010).

Local hyperthermia, or heat therapy, is recommended to be used once the phase of acute inflammation has subsided, which usually means 24 to 72 hours after injury or surgery (Dunning & Lascelles 2007). The benefits of heat therapy are similar to those of cryotherapy as it reliefs pain and provides muscle relaxation (Dunning & Lascelles 2007). Local hyperthermia can also increase the extensibility of articular or ligamentous collagen (Dunning & Lascelles 2007). The recommended duration of application is 15 to 20 minutes two to four times daily (Dunning & Lascelles 2007; Rychel 2010).

Passive range of motion exercises, that help to maintain the normal range of motion of joints, and therapeutic exercises such as walking on inclines, that help to strengthen the muscles that support the diseased joint and improve coordination in a controlled manner, may also be useful in the management of osteoarthritis (Dunning & Lascelles 2007; Schulz 2007).

Although there are currently only a few studies on the effect of exercise modifications on canine osteoarthritis, keeping the dog fit with regular and controlled exercise of moderate intensity is recommended as this helps to maintain the joint range of motion and muscle strength (Schulz 2007; Innes 2012). Swimming, for example, is an effective form of exercise for dogs with osteoarthritis (Taylor 2009). During the acute flares of osteoarthritis, a rest of a few days is recommended before the gradual return to exercise (Schulz 2007).

2.2.3. Nutritional supplementation

Nutritional supplements can be given to the animal as nutraceuticals and functional foods (Innes 2012). The term nutraceutical is used to describe a food or a part of a food that has medical or health benefits including the prevention and treatment of disease (Innes 2012; Vandeweerd et al. 2012). Functional foods are whole foods in which these supplements have been added (Innes 2012).

Glucosamine and chondroitin sulfate are among the most commonly used nutraceuticals although the evidence for their use varies (Rychel 2010; Innes 2012). Studies on the distribution of orally administered labeled chondroitin sulfate indicate that this nutraceutical might not reach the articular cartilage at all or at least not intact (Innes 2012). Thus based on the current evidence the use of chondroitin sulfate cannot be recommended for the management of canine osteoarthritis (Innes 2012). Slightly stronger evidence exists for the use of glucosamine in the management of osteoarthritis as it has been shown to increase proteoglycan synthesis in vitro and to have a weak anti-inflammatory effect in animal models (Innes 2012). However, clinical studies on using glucosamine alone in animals are needed before specific recommendations on its use can be given (Innes 2012). However, as neither of these nutraceuticals is associated with severe side-effects or absolute contraindications, their use is generally considered to be safe (Plumb 2011).

Other examples of nutraceuticals are essential fatty acids such as omega-3 fatty acid, avocado soybean unsaponifiable products, green-lipped mussel preparations, resin extract of the tree *Boswellia serrata* and vitamin C (Johnston et al. 2008; Rychel 2010; Innes 2012).

The problem with nutraceuticals is the lack of high quality studies on their efficacy in osteoarthritis (Vandeweerd et al. 2012). The systematic search of controlled clinical studies on the effect of nutraceuticals in horses, dogs and cats by Vandeweerd et al (2012) revealed only 25 studies that met the inclusion criteria. Five of these studies were performed in horses, sixteen in dogs and one in cats. According to this systematic review, omega-3 fatty acids were found to have the highest evidence of efficacy in the alleviation of clinical signs of osteoarthritis. For other reviewed nutraceuticals, such as hydroxycitric acid, P54FP, gelatine hydrosylate, β-1,3/1,6 glucans and special milk protein concentrate, the strength of evidence was evaluated to be low.

2.2.4. Other non-pharmacological treatment options

One of the alternative therapy options for pain management is acupuncture. It is being more commonly used in the pain management of veterinary patients (Henrotin et al. 2005; Skarda & Glowaski 2007). The exact mechanisms of action of medical acupuncture are not known, but the release of endogenous endorphins, local release of muscle spasm and decreased pain transmission to the spinal cord are likely to be involved (Rychel 2010). Although acupuncture seems to have potential as a treatment adjuvant, based on the current knowledge it should not replace the so-called Western treatments (Skarda & Glowaski 2007).

Examples of other alternative treatment options are homeopathy, chiropractic therapy and the use of herbal and plant medicines (Henrotin et al. 2005). However, inclusion of these therapy options to the treatment plan should be done cautiously as scientific evidence of their effects is scarce (Henrotin et al. 2005; Skarda & Glowaski 2007).

2.3. Surgical management

Pain that cannot be controlled by other means and the loss of limb function are indications for surgical management of osteoarthritis. Surgical treatment is a salvage procedure that is chosen in cases where other therapy options have failed (Aragon et al. 2007; Schulz 2007.)

Arthroplasty techniques, such as removal of femoral head and neck, joint replacement with prosthesis, surgical fusion of the joint with arthrodesis techniques or even amputation may be considered in patients whose symptoms do not respond to non-surgical treatment options (Schulz 2007).

Surgical management is also often indicated in treating the underlying orthopedic condition, such as cranial cruciate ligament rupture, if one can be identified (Schulz 2007).

INTRA-ARTICULAR INJECTIONS IN THE MANAGEMENT OF CANINE OSTEOARTHRITIS IN COMPARISON TO THEIR USE IN HORSES AND HUMANS

Intra-articular injections are less routinely used in the management of canine osteoarthritis compared to their use in horses and humans. The amount of scientific publications on their effects in dogs is also very scarce. Thus studies on the effects of intra-articular medications also on horses and humans are included in the review.

1. GENERAL PRINCIPLES OF INTRA-ARTICULAR INJECTIONS

1.1. Clinical use

- In veterinary medicine

Local intra-articular therapy is considered to be well suited to the management of osteoarthritis that is symptomatic only in a limited number of weight-bearing joints and lacks obvious systemic manifestations (Evans 2005; Larsen et al. 2008; Singh 2012).

In veterinary medicine intra-articular injections as a part of the management of osteoarthritis are mainly used in horses, in which species they play a significant role in the management of joint-related lameness (Caron 2005; Edwards 2011). Some of the reasons for this are the high prevalence of joint diseases in horses and the need to develop an alternative for NSAID therapy, that despite being an inexpensive and generally effective treatment option for reducing lameness in horses with osteoarthritis, is usually strictly regulated or even forbidden in competing horses and associated with potential side-effects (Caron 2005; Lamont & Mathews 2007; Edwards 2011). The pain-relieving effect of other systemic treatment options, such as nutraceuticals, is not comparable to that of the NSAIDs (Caron 2005). Another reason for the more frequent use of intra-articular injections in horses compared to the other veterinary species is that the lower limb joints of the horse are relatively easy to inject (Edwards 2011). Corticosteroids are the mainstay of intra-articular therapy in horses (McIlwraith 2011). The two other commonly used intra-articular agents are hyaluronic acid and polysulfated glycosaminoglycan (Edwards 2011).

Despite the wide-spread clinical use of intra-articular medications in the equine practice, the scientific basis for their use is not completely established (Clegg 2010). A problem with the current studies, in addition to their limited number, is that they have been mostly performed either *in vitro* or in animal models of osteoarthritis (Clegg 2010; McIlwraith 2011). The results from these studies might not be fully translatable into the treatment of naturally occurring disease (Clegg 2010).

The lack of high quality clinical studies on the effects of intra-articular medications in the management of osteoarthritis is a problem also in the canine practice, although the effects of certain intra-articular agents, such as corticosteroids and hyaluronic acid, have been studied in the canine joint (Pelletier & Martel-Pelletier 1989; Pelletier et al. 1994; Smith 2005b). Compared to their use in horses, intra-articular medications are considerably less routinely used in the canine practice. Some of the reasons behind this might be that their efficacy in the management of osteoarthritis is not fully established in any species and because arthrocentesis in dogs usually requires sedation.

- In human medicine

In human medicine the intra-articular injections are mainly used in the symptomatic treatment of knee osteoarthritis and they are considered as an additional treatment option in cases where other conservative treatment options have not been adequate in relieving the symptoms (Waddell 2007; Hameed & Ihm 2012; Keith 2012). Like in horses, corticosteroids and hyaluronic acid are the most commonly used intra-articular agents also in humans (Edwards 2011; Keith 2012).

As there are only few studies comparing the effects of hyaluronic acid directly to those of the corticosteroids, there are currently no definite recommendations regarding which of these two should be used as the initial injection (Keith 2012). Often intra-articular steroids are considered as the mainstay of injection therapy and hyaluronic acid as the second-line option (Zhang et al. 2008). Some authors suggest that corticosteroids might be more beneficial than hyaluronic acid in cases where the patient has an acutely inflamed osteoarthritic joint while hyaluronic acid might be more beneficial when the patient's condition is rather stable and the pain caused by

osteoarthritis is mild to moderate (Bannuru et al. 2009; Ara & Alam 2011; Hameed & Ihm 2012).

1.2. Advantages and disadvantages of using intra-articular drug delivery

- Advantages of the intra-articular drug therapy

The articular cartilage is avascular and alymphatic and as such is difficult to reach by using the systemic drug administration (Chevalier & Kemta-Lepka 2010; Kwon & Park 2012). Drugs from blood circulation reach the cartilage only by passive diffusion from the synovial fluid (Gege et al. 2012). Human studies suggest that the peak concentration of a drug after the oral administration is usually lower and is reached later in the synovial fluid than in plasma (Larsen et al. 2008). On the other hand, studies have also shown that after the steady state conditions have been achieved, the free NSAID concentrations are similar in synovial fluid and plasma (Larsen et al. 2008). The NSAID concentrations have also been shown to be more sustained in the synovial fluid than in plasma after oral or intravenous administration (Larsen et al. 2008)

To achieve a sustained therapeutic intra-articular drug concentration, high doses of a systemically given drug are often needed (Gege et al. 2012). This is not desired at all, as some of the currently available systemic treatment options are commonly associated with gastrointestinal, hepatic, renal and cardiac adverse effects, especially in older animals and people (Lamont & Mathews 2007; Singh 2012). One of the advantages of the use of intra-articular drug administration in joint diseases is the opportunity to deliver the drug directly to the cartilage while minimizing systemic exposure (Larsen et al. 2008; Edwards 2011; Kwon & Park 2012). Thus a smaller amount of drug is required for the desired pharmacological effect (Larsen et al. 2008). For certain drug candidates the local administration might even be the only realistic route of administration due to the severe systemic side effects with oral administration, low bioavailability or extensive degradation *in vivo* (Larsen et al. 2008; Chevalier & Kemta-Lepka 2010).

It is still unlikely, that local therapy would completely replace systemic therapies, even if an ideal medication was identified, since in some cases osteoarthritis is a

systemic disease with multiple joint involvement. Thus the need for better systemic treatment options still exists. (Singh 2012.)

- Challenges associated with intra-articular injections

The synovial cavity is not isolated but it is in direct equilibrium with circulation. The wide intercellular spaces and superficial capillaries in the synovium allow free flow of water and solutes so that the fluid volume in a joint cavity is replaced multiple times in a day. Thus the major reason for treatment failure following intra-articular drug administration is the rapid clearance of the drug from the joint space. (Edwards 2011.)

Small molecules, such as the cytokines and most drugs including the corticosteroids, NSAIDs and local anesthetics, diffuse through the synovial lining with ease (Edwards 2011). Thus for many drugs the absorption and redistribution into the systemic circulation following an intra-articular injection are actually comparable to other non-intravenous parenteral routes (Edwards 2011). Often this clearance from the synovial cavity is further accelerated by synovitis (Kwon & Park 2012). Thus, the biggest challenges in using intra-articular drug delivery are keeping the drug concentration stable in the joint cavity and preventing the drug from leaving the joint space and distributing throughout the body (Edwards 2011; Kwon & Park 2012).

The desired drug concentration could be maintained over extended periods of time by repeated intra-articular administrations, but repeated intra-articular injections are neither feasible nor safe due to the increased risk of infection (Evans 2005; Larsen et al. 2008). In addition to this, since dogs are recommended to be sedated or anesthetized for intra-articular injections, in canine practice repeated intra-articular injections would also mean repeated sedations. More ideally the stable drug concentration in the synovial cavity would be achieved by immobilizing the active agent into an injectable depot formulation from which it would be released in a controlled manner (Larsen et al. 2008). For this reason current studies revolve around creating sustained-release drug formulations (Edwards 2011; Kwon & Park 2012).

The accurate needle placement obviously affects the efficacy and safety of intra-articular medications and may be particularly problematic in conscious patients (Edwards 2011; Smart 2012). This is not a problem only in veterinary medicine but also in human medicine since while rheumatologists may achieve full accuracy, it has been

estimated that in hospital settings almost one-third of the knee injections may actually not even reach the joint cavity (Edwards 2011). This happens despite the fact that the joint cavity of human knee joint is relatively large and accessible compared to that of the stifle joint of many of the dog breeds (Edwards 2011).

In humans the accuracy of needle placement has been recommended to be confirmed by the return of synovial fluid during the joint aspiration (Smart 2012). Inspecting the gross appearance of synovial fluid before injecting the drug is also advisable, as purulent effusion on aspiration is clearly a contraindication for intra-articular medications (Philipose et al. 2011).

The amount of synovial fluid in chronically affected joints might be so reduced that no synovial fluid can be aspirated before the injection, which makes it difficult to be assured that the needle is accurately placed in the synovial cavity (Smart 2012). In these cases the accuracy of the injection can also be improved by using imaging such as ultrasonography as an aid, as is often done in human medicine when injecting the joints of hands (Edwards 2011; Iannitti et al. 2011). In dogs arthrography has also been used to confirm the correct needle placement (Hadley et al. 2010).

Achieving a correct diagnosis of an intra-articular disease is also important, as an intra-articular medication lacks efficacy also in cases where the pain actually stems from the soft tissues surrounding the joint (Edwards 2011).

- Potential risks associated with intra-articular injections

The use of intra-articular medications is not completely free from potential adverse effects. In humans some of the reported complications associated with intra-articular injections are infection, post-injection flare, crystal-induced synovitis, cutaneous atrophy and steroid arthropathy (Iannitti et al. 2011; Kwon & Park 2012). Although these side effects are rare, introducing bacteria into the joint cavity is a major concern, because the consequences of septic arthritis can be devastating (Edwards 2011; Iannitti et al. 2011; Kwon & Park 2012). The risk of septic arthritis is considered to be especially high when using intra-articular corticosteroids that have the potential to suppress the inflammatory response to microbes (Singer 2008; Edwards 2011).

The prevalence of septic arthritis following intra-articular injections in dogs has not been investigated, but the overall incidence of septic arthritis, whether arising from

the penetration of the joint capsule due to surgery or trauma, hematogenous spread or spread from adjacent tissues, is low in the canine practice (Ridge 2011). The incidence of septic arthritis following elective arthroscopic surgery was reported to be 0,85 % in 294 dogs and 11 cats in the retrospective study by Ridge (2011). The incidence of septic arthritis is low also in humans, as the prevalence of joint infection associated with intra-articular corticosteroid injections has been reported to be around 4 cases in every 10 000 injections (Mathews 2010). The risk of septic arthritis can be reduced by using strict aseptic technique as described below and by abstaining from injecting intra-articular medications in cases where there are signs of a systemic or local infection (Caron 2005; Hameed & Ihm 2012).

In some cases the drug itself can be the cause of the inflammatory response, because the synovium is highly reactive (Edwards 2011). For example, in some horses, saline and hyaluronic acid injections as such have been shown to evoke a marked inflammatory response and to cause lameness (Edwards 2011). It is often challenging to differentiate reactive arthritis from septic arthritis, because of the similarities in the clinical signs and even in the findings on cytology of synovial fluid (Singer 2008). As the consequences of septic arthritis can be severe, it is often safer to start the treatment as if the cause of inflammation is infectious whenever acute lameness follows an intra-articular injection (Singer 2008).

1.3. Injection technique

The injection site should be aseptically prepared, which includes the clipping off the hair and the surgical scrubbing and disinfecting of the skin (Fossum 2007; Taylor 2009) Sterile gloves and single-use syringes and needles should be used to reduce the risk of introducing bacteria into the joint (Caron 2005). Recommended sites for arthrocentesis in dogs are shown in Figure 2.

The literature recommends the use of 25-gauge needles for arthrocentesis in small dogs and 22-gauge needles for arthrocentesis in larger dogs (Taylor 2009). However, it can be difficult to aspirate synovial fluid through a small needle as it is very viscous. Therefore, 21-gauge needles are commonly used for arthrocentesis in large dogs and 23-gauge needles for arthrocentesis in small dogs and cats (Helka Heikkilä, personal communication). Because of the thick musculature, reaching the hip joint of large dogs

might even require using a 3-inch spinal needle (Taylor 2009). The needle should be attached to the syringe before it is inserted into the joint and as stated above, the correct needle placement should be verified before injecting the medication (Clements 2006). In human medicine, some practitioners remove the excessive effusion fluid before the injection, as this not only helps to reduce the concentration of inflammatory mediators in the joint cavity, but also makes sure that the injected medication will not be excessively diluted (Strauss et al. 2009). Removing the excessive synovial fluid might also relieve pain that is caused by the stretching of the joint capsule (Liebich et al. 2007). After the injection any negative pressure on the syringe is released before the withdrawal of the needle (Taylor 2009).

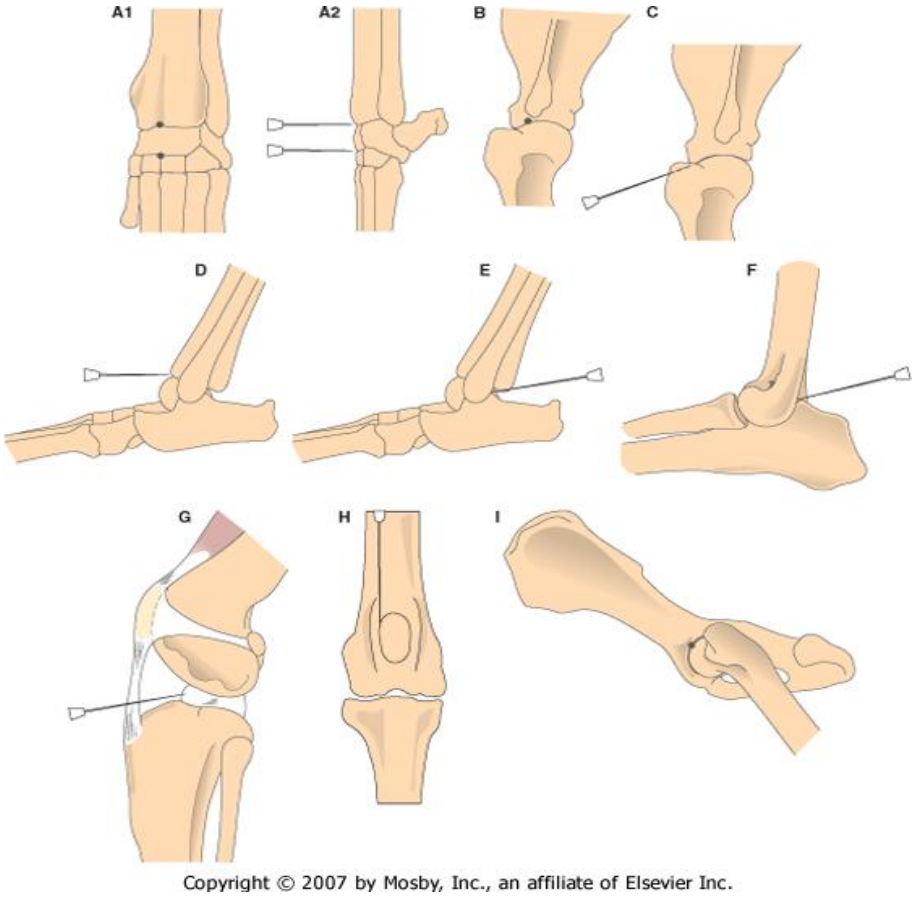

Figure 1. Recommended sites of arthrocentesis in dogs (Fossum 2007).

2. OVERVIEW OF THE MEDICATIONS FOR INTRA-ARTICULAR THERAPY OF OSTEOARTHRITIS

The following list of medications used or studied in the intra-articular treatment of osteoarthritis is not exhaustive. The focus of the review is on corticosteroids and hyaluronic acid, because they are the most commonly used intra-articular medications in both human and veterinary medicine. Some of the new therapy options with potential clinical use in the near future will also be discussed.

2.1. CORTICOSTEROIDS

2.1.1. Mechanism of action

Despite their popularity, the exact mechanism by which corticosteroids affect the osteoarthritic joint is not completely known, but their effect is thought to be mostly mediated through the inhibition of the activity of phospholipase A leading to reduced production of both cyclooxygenases and lipoxygenases (Schulz 2007; Gege et al. 2012). Intra-articular steroids reduce the number of inflammatory cells such as lymphocytes, macrophages and mast cells which in turn decreases phagocytosis, lysosomal enzyme release and the release of inflammatory mediators (Lavelle et al. 2007). Corticosteroids also inhibit a number of cytokines and enzymes involved in the articular cartilage degeneration in osteoarthritic joints (Caron 2005; Lavelle et al. 2007). They have been shown to reduce the release of leukotrienes and prostaglandins and the expression of two of the most important mediators of cartilage degradation, interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) (Caron 2005; Lavelle et al. 2007). Corticosteroids might also protect the cartilage by reducing metalloproteinase activity (Schulz 2007).

2.1.2. Scientific evidence and clinical use

- In veterinary medicine

The use of corticosteroids has a long history in veterinary medicine since the report on the use of hydrocortisone in the treatment of different musculoskeletal conditions in horses and cattle was released by Wheat et al. already in 1955 (McIlwraith 2010).

Despite this, almost all of the studies on the effects of corticosteroids in the management of canine or equine osteoarthritis have been performed *in vitro* or on animal models.

The results from these studies have also been rather contradictory (Hossain et al. 2008; Innes 2012). On the one hand some protective function of intra-articular corticosteroids on articular cartilage has been demonstrated in experimental canine models, but on the other hand undesired effects, such as increased apoptosis and suppressed proliferation of chondrocytes, have also been demonstrated in *in vitro* studies (Pelletier et al. 1994; Hossain et al. 2008).

The only controlled prospective studies currently available on the effects of intra-articular corticosteroid injections on osteoarthritis in dogs date back to the late 1980s and early 1990s (Pelletier et al. 1989; Pelletier & Martel-Pelletier 1991; Pelletier et al 1994; Pelletier et al. 1995). However, these studies investigate the effect of corticosteroids on the histological and macroscopic severity of osteoarthritis rather than on the clinical signs.

The effect of intra-articular triamcinolone hexacetonide was studied by Pelletier et al. (1989). In this study osteoarthritis was induced for twenty-four dogs by the severance of the cranial cruciate ligament. The treatment group consisted of twelve dogs of which six dogs received oral prednisolone and six dogs received 5 mg of triamcinolone hexacetonide intra-articularly at the time of the induction of osteoarthritis and four weeks later. The control group consisted of twelve dogs that received no treatment. The macroscopic changes, such as osteophyte formation and changes in the gross appearance of articular cartilage, as well as the histological changes attributable to osteoarthritis were shown to be less severe in the treatment group compared to those in the control group. Also, no evidence of increased cell degeneration or cell death caused by corticosteroids was noted.

In another study by Pelletier et al. (1994) osteoarthritis was induced for fifteen dogs also by severing the cranial cruciate ligament. The treatment group consisted of eight dogs that received an intra-articular injection of 20 mg of methylprednisolone acetate into the stifle joint at the time of the induction of osteoarthritis and four weeks later. The control group consisted of seven dogs that did not receive any treatment. The results showed that the osteophyte formation and the histologic signs of osteoarthritis in the articular cartilage, such as fibrillation and fissure formation of the cartilage

surface, were significantly less severe in the dogs of the treatment group compared to those in the control group.

In a small patient series by Kinzel et al. (2003) intra-articular triamcinolone acetonide (10 mg/joint) was used with lidocaine in the treatment of osteoarthritic pain in the cervical facet joints of client-owned Scottish Deerhounds. Eight out of nine dogs responded to the treatment and seven of the dogs were painless for more than four months. Although the results of this study were interesting, they should be interpreted with care since this was a retrospective study with no control group and possibly with some selection bias in the inclusion criteria.

Intra-articular corticosteroids are commonly used in the treatment of equine osteoarthritis, although the scientific evidence on their efficacy in equine osteoarthritis is scarce (McIlwraith 2011). Most of the current studies in horses are either *in vitro* studies or performed on animal models of osteoarthritis (McIlwraith 2010).

An example of a study on horses with naturally occurring osteoarthritis is the retrospective study by Labens et al. (2007). Forty-eight horses with osteoarthritis in the distal tarsal joints were treated with methylprednisolone acetate or triamcinolone acetonide, either alone or combined with hyaluronic acid. Although the grade of lameness improved in approximately sixty percent of the treated limbs, about ninety percent of the horses were lame again at the second control visit that was done a median of 56 days after the first examination. In a retrospective case series by Smith et al. (2005a) no pain relief after intra-articular methylprednisolone acetate or triamcinolone hexacetonide injection was noted in horses with talocalcaneal osteoarthritis.

In dogs corticosteroid injections are less commonly used than in horses as they are usually reserved for cases where severe end stage osteoarthritis does not respond to other treatment options and the animal is suffering considerably (Henrotin et al. 2005). Systemic corticosteroids are not used in the management of human osteoarthritis and they are not recommended to be used in the treatment of canine osteoarthritis, either (Schulz 2007; Ara & Alam 2011).

In horses intra-articular corticosteroids are commonly used in combination with hyaluronic acid which might be protective against the side effects of corticosteroids and allow the reduction of the dose of corticosteroids (van Wereen & de Grauw 2010; McIlwraith 2011). Despite this practice being based more on tradition rather than

scientific evidence, it is quite popular among equine practitioners (van Wereen & de Grauw 2010; McIlwraith 2011).

- In human medicine

In human medicine intra-articular corticosteroid injections have been routinely administered to treat joint pain since the 1950s (Lavelle et al. 2007). In the treatment of human osteoarthritis intra-articular corticosteroids are generally used as a treatment adjunct after the initial recommended treatment options such as the non-pharmacological and pharmacological therapies, mostly NSAIDs, have been tried and found inadequate (Douglas 2012).

In humans, the scientific evidence on the efficacy of intra-articular corticosteroid injections in the treatment of osteoarthritis is strong (Cheng et al. 2012). However, corticosteroids have been shown to be most effective in those joint problems where inflammation is the most important component of the disease such as rheumatoid arthritis and juvenile idiopathic arthritis (Habib et al. 2010). In most cases of osteoarthritis the articular degeneration is a more important component of the disease than inflammation (van Wereen & de Grauw 2010). Thus corticosteroids are not recommended to be used as the sole therapy for patients with chronic and stable osteoarthritis (Ara & Alam 2011; Douglas 2012). The long-term benefits of corticosteroids have also not been confirmed (Colen et al. 2010; Kon et al. 2012). However, corticosteroids can be very useful in the acute exacerbations of osteoarthritis that are seen as local inflammation with joint effusion as they have the potential to suppress the inflammation rapidly and effectively (Ara & Alam 2011; Douglas 2012).

This effect, however, seems to be only temporary. According to the meta-analysis of trials comparing hyaluronic acid with corticosteroids in the management of human osteoarthritis performed by Bannuru et al. (2009) corticosteroids appear to be more effective for pain reduction up to 4 weeks after treatment, but by and after 8 weeks the relative effects of hyaluronic acid products were shown to be greater.

2.1.3. Dosing

Generally, the long-acting corticosteroid preparations, such as methylprednisolone acetate, triamcinolone acetonide and triamcinolone hexacetonide, are preferred for the intra-articular use (Innes 2012). Of these, methylprednisolone acetate (Depo-Medrol®) is licenced for intra-articular use in dogs in Finland (Lääketietokeskus 2013). In addition to methylprednisolone acetate, also triamcinolone acetonide has an approval from the United States Food and Drug Administration (FDA) for the intra-articular use in dogs (Plumb 2011; FDA 2013).

In the aforementioned study on an experimental model of canine osteoarthritis by Pelletier et al (1994) a single injection with 20 mg of methylprednisolone acetate resulted in favorable results in the structural changes of osteoarthritis. This is also the FDA-approved dose and the dose recommended for a large joint space by the manufacturer of Depo-Medrol vet® (FDA 2013; Lääketietokeskus 2013). Although methylprednisolone acetate is used in the intra-articular treatment of osteoarthritis of the knee in human medicine, in equine practice methylprednisolone acetate is generally not recommended to be used in high-motion joints such as the stifle and carpal joints, due to its potential harmful effects on articular cartilage demonstrated in equine studies (McIlwraith 2010; McIlwraith 2011; Cheng et al. 2012).

The FDA-approved dose for the intra-articular administration of triamcinolone acetonide in dogs is 1,0-3,0 mg as a single injection, although it can be repeated after 3 to 4 days if clinical signs are severe or the clinical response is poor (FDA 2013). Triamcinolone acetonide is commonly used in equine practice and it is recommended for the use in high-motion joints (McIlwraith 2011; Plumb 2011).

In the study by Pelletier et al. (1989) the dose for triamcinolone hexacetonide was 5 mg and the injection was repeated after four weeks. Triamcinolone hexacetonide is not currently approved in Finland or by FDA for the intra-articular use in canine osteoarthritis. However, in a review on the knee injections for the treatment of human osteoarthritis by Cheng et al. (2012), triamcinolone hexacetonide was considered more effective than triamcinolone acetonide.

Although there are numerous publications on the use of intra-articular corticosteroids in the knee osteoarthritis in human medicine, the amount and quality of the current data is not considered to be enough for drawing any conclusions about

the most efficacious agent, or the dose and dosing frequency (Douglas 2012). There are also only few randomized controlled studies directly comparing the efficacy of different intra-articular corticosteroids between each other (Douglas 2012). The problem is the same in the equine practice (McIlwraith 2010). The current usage patterns are therefore largely determined by the opinions of the practitioners (Douglas 2012). There are currently no official guidelines for the dosages of intra-articular corticosteroids for the use in canine osteoarthritis, either.

Some recommendations regarding the injection frequency of intra-articular corticosteroids exist in humans and the same guidelines have been thought to be adaptable also to small animal medicine (Innes 2012). As there is concern about the potential harmful effects of intra-articular corticosteroids on the articular cartilage, a sufficient time period, such as at least 6-12 weeks, is generally recommended be left between the injections of the same joint and the same joint should not be injected more than 2-4 times a year (Fox & Stephens 2010; Douglas 2012; Innes 2012). On the other hand, some authors do not recommend to repeat an injection if the first one did not demonstrate any effect (Douglas 2012).

2.1.4. Potential risks

The intra-articular use of corticosteroids is also associated with potential side-effects (Douglas 2012; Innes 2012). Among the most notable of these is their potentially destructive effect on articular cartilage as intra-articular corticosteroids have been shown to depress chondrocyte metabolism and diminish proteoglycan and collagen synthesis (Schulz 2007; Douglas 2012; Innes 2012; Kon et al. 2012). As mentioned earlier, especially the safety of methylprednisolone acetonide has been questioned based on studies in horses (McIlwraith 2010; McIlwraith 2011). On the other hand, some evidence exists that lower dosages of corticosteroids might actually be chondroprotective and delay the progression of cartilage lesions (Kon et al. 2012). An elevated risk of weakening of the ligaments of the injected joint has also been associated with repeated intra-articular corticosteroid injections (Colen et al. 2010). Thus, in humans corticosteroids are not recommended to be injected into joints with instability (Philipose et al. 2011).

An elevated risk of infection, especially with repeated injections, has also been associated with corticosteroid injections (Colen et al 2010). To reduce the risk of introducing pathogens into the joint cavity, strict aseptic technique must be followed like in all cases of intra-articular injections (Innes 2012). In addition to this some equine practitioners routinely use intra-articular antibiotics with corticosteroid injections (van Wereen & de Grauw 2010). Systemic bacteremia, suspected septic arthritis or infection of the overlying soft tissues are absolute contraindications for intra-articular corticosteroid injections (Lavelle et al. 2007; Philipose et al. 2011).

Intra-articular corticosteroids can cause synovitis without microbial infection. This reactive synovitis following a corticosteroid injection, also known as the steroid flare, has been documented in horses and humans with a prevalence of 2-6% in humans (Lavelle et al. 2007; Edwards 2011). It is believed to be a form of chemical synovitis caused by the injected crystals (Lavelle et al. 2007).

There are reports on human patients that the absorption of intra-articularly administered corticosteroids into systemic blood circulation has resulted in systemic side-effects (Habib 2009). As intra-articular corticosteroids have been shown to cause an increase in the blood glucose levels for a few days after the injection, some concern has been raised regarding their use in patients with diabetes (Lavelle et al. 2007; Habib 2009). Intra-articular steroids have also been shown to affect the hypothalamic-pituitary-adrenal axis transiently resulting in a reduction in serum cortisol levels that typically normalize in a few days (Lavelle et al. 2007). It is also noteworthy, that the use of corticosteroid injections is contraindicated in pregnant animals (FDA 2013, Lääketietokeskus 2013).

Despite the potential risks, most human studies on corticosteroids indicate that when used judiciously their benefits exceed the potential risks (Innes 2012). However, clinical studies specially focusing on the progression of osteoarthritis after corticosteroid injection are still needed (Kon et al. 2012).

2.2. HYALURONIC ACID

2.2.1. Mechanism of action

Hyaluronic acid (HA, hyaluronan) is a polysaccharide that consists of a long chain of disaccharides (β-D-glucuronyl- β-D-N-acetylglucosamine) (Henrotin et al. 2005).

Endogenous hyaluronic acid produced by the type B synoviocytes and fibroblasts has an important function as it is largely responsible for the shock-absorbing and lubricating properties of the synovial fluid (Henrotin et al 2005; Bannuru et al. 2009; Gomis et al 2009; Kwon & Park 2012).

Intra-articular hyaluronic acid injections are widely used in human medicine despite the fact that their mechanism of action is not completely understood (Evans 2005). In osteoarthritis the concentration and molecular weight of hyaluronic acid are markedly reduced due to fragmentation and insufficient production (Henrotin et al. 2005; Venable et al. 2008; Gomis et al. 2009; Kwon & Park 2012). This interferes with the physiological functions of synovial fluid as the decreased lubrication increases the stress upon the diseased cartilage leading to further cartilage damage (Kwon & Park 2012). Viscosupplementation with exogenous hyaluronic acid is aimed at restoring the viscoelasticity of synovial fluid and reducing the pain associated with the joint movement (Henrotin et al. 2005; Larsen et al. 2008; Gomis et al. 2009; Kwon & Park 2012).

In addition to the mere restoration of the elastic and viscous properties of synovial fluid, studies indicate that hyaluronic acid might also have other mechanisms of action and identifying other biological properties of hyaluronic acid has been attempted (Evans 2005; Abate et al. 2012). Potential anti-inflammatory, anti-nociceptive and chondroprotective properties of hyaluronic acid have been suggested, although mostly based on *in vitro* studies (Colen et al. 2010; Edwards 2011).

Possible chondroprotective modes of action in addition to restoration of the synovial fluid elastoviscosity include the promotion of endogenous hyaluronic acid production, stimulation of chondrocyte matrix component synthesis and inhibition of chondrocyte matrix metalloproteinase synthesis (McNeil 2011). The anti-inflammatory effect is thought to be caused by decreased migration of inflammatory cells and lowered levels of inflammatory mediators such as prostaglandin E_2 and bradykinin. (Abate et al. 2012) *In vitro* studies also suggest that hyaluronan might enhance cartilage matrix synthesis, blunt responses to IL-1, prevent damage caused by oxygen-derived free radicals, inhibit phagocytosis and protect chondrocytes from apoptosis (Evans 2005; Schulz 2007). Hyaluronic acid of higher molecular weight has also been shown to inhibit the hyaluronic acid degradation (Caron 2005; Abate et al. 2012;Kwon & Park 2012).

The analgesic effect of intra-articular hyaluronan has been proposed to be caused at least partly by the inhibition of the pain receptors, reduction of the sensitivity of synovial nerve endings and stimulation of synovial lining cells (Caron 2005; Evans 2005; Abate et al. 2012).

It is yet to be explained why the maximal clinical effect of hyaluronic acid occurs several weeks after the injection and persists for a relatively long time, despite the fact that the injected hyaluronic acid is cleared from the joint space in less than a day (Evans 2005; Colen et al. 2010). One of the theories to explain this is that the supplemented hyaluronic acid induces the synthesis of the endogenous hyaluronic acid (Evans 2005). Although the half-life of hyaluronic acid in the joint space is remarkably shorter than its duration of clinical effect, hyaluronic acid is currently still classified as a viscosupplement and not as a biologic therapy (Waddell 2007; Schulz 2007; Edwards 2011).

There are several different hyaluronic acid preparations of different molecular weight, concentration and origin commercially available (Evans 2005). Common sources of exogenous hyaluronic acid are the rooster comb and bacterial fermentation (Evans 2005; Kon et al. 2012). The molecular weight of the commercially available hyaluronic acid preparations is generally somewhat smaller than that of the endogenous hyaluronic acid (Edwards 2011). Thus a cross linked hyaluronic acid preparation with an increased viscosity and longer half-life, known as hylan, has been developed (Evans 2005).

2.2.2. Scientific evidence and clinical use

- In veterinary medicine

The first clinical trials on intra-articular hyaluronic acid were performed on racehorses when Butler et al in 1970 demonstrated that horses performed better after the hyaluronic acid injection (Evans 2005). Hyaluronic acid is still commonly administered intra-articularly for the management of osteoarthritis in horses although clinical studies on the effect of hyaluronic acid on horses with naturally occurring osteoarthritis are currently lacking (Edwards 2011; McIlwraith 2011).

The scientific evidence on the efficacy of intra-articular hyaluronic acid in the treatment of naturally occurring osteoarthritis in dogs is scarce. The studies performed on experimental canine models of osteoarthritis have failed to demonstrate clear benefits of hyaluronic acid supplementation (Aragon et al. 2007; Johnston et al. 2008; Sanderson et al. 2009). On the other hand, the quality of these studies has also been evaluated to be rather low in two systematic reviews (Aragon et al. 2007; Sanderson et al. 2009).

Hellström et al. (2003) compared the efficacy of intra-articular high molecular weight sodium hyaluronate to that of oral carprofen in 36 dogs with naturally occurring osteoarthritis. The dogs received two injections of sodium hyaluronate three weeks apart. At six weeks the lameness of the dogs had significantly improved compared to the carprofen group. However, the response to treatment was evaluated only by visual examination of lameness and no objective outcome variables were used.

Smith et al. (2005b) performed a study that was designed to determine whether intra-articular hyaluronan injections alter the progression of osteoarthritis and pain perception after the transection of anterior cruciate ligament in a canine model of osteoarthritis. The 30 dogs that were included in the study were divided into three study groups that received intra-articular injections once a week for the first five weeks after surgery and again for five weeks 13 weeks after surgery. The prophylactic group received hyaluronan during the first injection series and saline during the second series while the treatment group was given saline in the first series and hyaluronan in the second series. The control group received saline during both sets of injections. No significant differences were noted between the study groups neither in the ground reaction forces measured with a force platform, the arthroscopic examination 12 weeks after ligament transection or on the gross examination 32 weeks after ligament transection. Histologic scores and biochemical composition of articular cartilage were also similar between the three groups.

The results from a previous study by Smith et al (2001) were also discouraging. The anterior cruciate ligament of the left stifle joint of 14 dogs was transected and the dogs were then divided into two groups of seven dogs. In the treatment group the unstable knee was injected with 10 mg of hyaluronic acid once a week for five weeks starting the day after surgery, while in the control group the knee was injected with saline. The analysis of synovial fluid revealed no changes in the synovial fluid volume or in the

molecular weight of hyaluronic acid. The administration of hyaluronic acid also did not restore the synovial fluid hyaluronic acid concentration to normal levels.

More promising results were obtained from a study by Echigo et al. (2006) on the effect of intra-articular hyaluronic acid on the apoptotic chondrocytes in the articular cartilage after the experimental cranial cruciate ligament rupture in eight dogs. The number of apoptotic chondrocytes in the articular cartilage was found to be lower in the dogs treated with hyaluronic acid compared to the eight dogs in the control group.

The results from the placebo-controlled study by Wenz et al. (2000) were also encouraging. After the induction of osteoarthritis of twenty-seven dogs by the severance of the cranial cruciate ligament, the nine dogs in the treatment group were divided into subgroups that received four injections of hyaluronan intra-articularly once a week beginning either three, six or twelve weeks after the surgery. The specimens of articular cartilage were collected five weeks after the last injection and examined both macroscopically and histologically. There were found to be significantly less changes in the articular cartilage attributable to osteoarthritis in the joints treated with hyaluronic acid compared to the placebo-treated joints. The injected dose of hyaluronic acid was two times larger in this study than in the studies by Smith et al (2001 and 2005b).

Hyaluronic acid has been shown to possess potential beneficial effects on the articular cartilage also in other experimental models. One of these is the study by Sagliyan et al. (2009) on the effect of using hyaluronic acid with autogenic cancellous grafts in the treatment of experimentally induced osteochondral defects in the canine stifle joints. A defect of 10 mm in depth was created on the femoral sulcus of both legs of 10 dogs and filled with autogenic cancellous graft. The left stifle joint was injected with 2 mg/kg of hyaluronic acid immediately after the operation and 1 month afterwards. The right stifle joints served as the control group. Half of the dogs were sacrificed 3 months and the rest 6 months after the surgery and the joints were evaluated macroscopically and histologically. In the joints injected with hyaluronic acid ossification process and trabeculous bone formation seemed to occur faster than in the control joints.

The use of hyaluronic acid in the management of canine osteoarthritis is not even nearly as established as it is in the treatment of equine osteoarthritis. In horses, hyaluronic acid as an only intra-articular medication is recommended mostly for cases

in which the synovitis is mild to moderate. However, it is usually combined with an intra-articular corticosteroid, as this provides a faster and more effective response to treatment. Another benefit of the combined use is that hyaluronic acid might decrease the risk of potential side effects of intra-articular corticosteroids. (McIlwraith 2011).

- In human medicine

The first studies on the use of hyaluronic acid in the management of human knee osteoarthritis were carried out in the 1970s (Migliore & Granata 2008). Unlike in dogs, there are clinical studies and meta-analyses confirming the efficacy of hyaluronic acid in the management of human patients with osteoarthritis (Edwards 2011; Iannitti et al. 2011). For example, in a study performed by Huang et al (2011) five weekly injections of 500-730 kDa sodium hyaluronate resulted in significantly greater improvement in the pain score from baseline to week 25 in two hundred human patients with knee osteoarthritis compared to a placebo group.

On the other hand, contradictory results have also been reported and the conclusions of certain clinical studies and meta-analyses have varied between dramatic improvements to no beneficial effect (Evans 2005; Colen et al 2010). The placebo effect and the differences between different hyaluronic acid preparations may explain some of the discrepancies found in the literature (Evans 2005). The blinding is also particularly challenging when injecting hyaluronic acid because it is easily recognized due to its high viscosity (Evans 2005). Thus it is recommended, that in the studies on the effects of hyaluronic acid, the treating physician and the outcome observer are not the same person (Evans 2005).

Currently intra-articular hyaluronic acid therapy is recommended for those human patients that have not responded to standard non-pharmacologic and pharmacologic treatment options or that have contraindications for NSAID use or surgical treatment (Waddell 2007; Kwon & Park 2012). Compared to intra-articular corticosteroids, hyaluronic acid is usually considered as the second-line option in cases where corticosteroid injections have not provided adequate treatment response (Kwon & Park 2012). There is some evidence that the pain-relieving effect of hyaluronic acid is longer than that of the corticosteroids but corticosteroids are still preferred over hyaluronic acid in cases of acute inflammation (Waddell 2007; Ara & Alam 2011; Kwon

& Park 2012). One should also bear in mind that the hyaluronic acid preparations are a lot more expensive than corticosteroids (Ara & Alam 2011).

Despite the lack of firm conclusions about the clinical efficacy of intra-articular hyaluronic acid injections, the treatment continues to be very widely used in humans in the absence of competing options (Evans 2005).

2.2.3. Dosing

There are no official guidelines on dosing and frequency of administration for the use of intra-articular hyaluronic acid in dogs. Also, currently none of the hyaluronic acid products on the market in Finland or in the United States has an approval for the intra-articular use in canine osteoarthritis (Lääketietokeskus 2013; Plumb 2011).

In the aforementioned studies by Smith et al (2005b) and Smith et al (2001) on the experimental canine models of osteoarthritis, the dose of hyaluronic acid was 10 mg given to dogs once a week for 5 consecutive weeks. The dogs in these studies weighed 20-30 kg. However, as stated earlier, the results from these studies were not particularly encouraging. In the study by Wenz et al. (2000) the dose of hyaluronic acid was 20 mg given to the stifle joints of foxhounds weighing 26-32 kg.

When used as an adjunctive treatment for canine synovitis, the recommended dose has been remarkably lower, as only 3-5 mg of high molecular weight hyaluronan at weekly intervals has been suggested (Plumb 2011).

An example of a recommended clinical treatment regime in horses is a series of four to five hyaluronan injections at 7- to 14-day intervals (Caron 2005). In the treatment of human knee osteoarthritis hyaluronic acid is typically given once a week for 3-5 weeks (Evans 2005; Strauss et al. 2009).

2.2.4. Potential risks

Intra-articular hyaluronic acid is commonly used and safe and there are no specific contraindications for its use, other than those for intra-articular injections in general (Baltzer et al 2009; Plumb 2011). No adverse effects were mentioned in the aforementioned studies on dogs, either (Smith et al. 2001; Smith et al. 2005b).

Most of the reported side effects have been local, for example local heat, swelling and effusion (Plumb 2011; McNeil 2011). Usually these symptoms subside within 24-48

hours and require no treatment (Plumb 2011). In addition to these, allergic reactions have also been reported rarely in humans (Evans 2005). The incidence of adverse effects has been reported to be more common when using chemically cross-linked preparations (hylan) (Evans 2005; McNeil 2011).

2.3. THERAPIES TARGETED AT THE BIOLOGICAL MECHANISMS OF OSTEOARTHRITIS

As the role of biological mechanisms in the pathogenesis of osteoarthritis was recognized only three decades ago, the idea of affecting the biologic mechanisms involved in osteoarthritis is also relatively new. Only in the last decade has there been any serious attempt to develop therapy options to affect the cellular mechanisms of the disease process. (Evans 2005.)

The goal in the field of biologically based therapies is to develop a treatment modality with regenerative action that would provide both anabolic and anti-catabolic activities (Textor 2011). Developing means to affect the receptors and signaling pathways of the cytokines and growth factors involved in the disease process or to control directly the expression of the responding genes is also under research (Evans 2005).

The use of cytokine inhibitors and growth factors in the treatment of osteoarthritis was first proposed in the late 1970s and early 1980s (Wehling et al. 2007). The rationale behind these therapies is based on the current opinion that there is an imbalance in the production of the pro-inflammatory and anti-inflammatory cytokines (Sampson et al. 2010). Thus, therapies that would reduce the effects or production of the pro-inflammatory cytokines, especially IL-1 and TNF-α, or that would be a source of anabolic factors such as growth factors, could potentially alter the course of the disease (Evans 2005; Calich et al. 2010; Fox & Stephens 2010).

Currently the anti-cytokine treatment options targeted at IL-1 are the administration of interleukin-1 receptor antagonist (IL-1Ra) and Diacerein (Calich et al. 2010). IL-1Ra can be administered either via autologous blood products, such as autologous conditioned serum, or as a synthetic form, known as anakinra that is used in the treatment of rheumatoid arthritis in humans (Evans 2005; Calich et al. 2010; Textor 2011). IL-1Ra has also been administered through gene transfer in an equine

model of osteoarthritis and in human medicine (Frisbie et al. 2007). Diacerein is not administered intra-articularly and the use of anakinra has not been studied on dogs, so they will not be discussed here. Adalimumab, which is an inhibitor of TNF-α, will also be left out of the review due to the lack of studies in veterinary medicine.

The main ways of growth factor administration are currently autologous blood injection (ABI) and platelet-rich plasma preparations (Creaney et al. 2011). As studies on the use of whole blood in the management of osteoarthritis are lacking, only the use of platelet-rich plasma will be discussed in the following section.

The use of autologous blood products has been increasing as they might provide cellular and humoral mediators to enhance healing in tissues with low healing potential (Filardo et al. 2012). There are several methods of concentrating blood derived growth factors and other potentially beneficial cytokines including platelet rich plasma (PRP), autologous conditioned serum (ACS) and autologous blood injection (Fox & Stephens 2010; Leong et al. 2012). Although PRP and ACS are both derived from whole blood, their mechanisms of action are different (Textor 2011). The differences between these two methods will be discussed later.

The clinical use of autologous blood products both in veterinary and human medicine has already started. However, one should note that as the use of autologous biologic therapies is not limited by the restrictions and testing required for pharmaceuticals and as their use in general is very safe, it has been suggested that enthusiasm might have outpaced the scientific evidence of their effects. (Stief et al. 2011.)

2.3.1. Mesenchymal stem cells

2.3.1.1. Mechanism of action

Stem cells are defined as undifferentiated cells with the ability to convert into differentiated cells and to regenerate tissues (Mafi et al. 2011; Fortier & Tuan 2012). The three main classes of stem cells are the embryonic stem cells, fetal stem cells and adult stem cells (Fortier & Tuan 2012). The adult stem cells, such as mesenchymal, neural and hematopoietic stem cells, are responsible of the normal tissue maintenance (Mafi et al. 2011; Punwar & Khan 2011; Gattegno-Ho et al. 2012; Fortier & Tuan 2012).

Currently most of the clinical attention has focused on the mesenchymal stem cells (MSCs) that have the potential to differentiate into the cells of mesenchymal origin, such as chondrocytes, osteoblasts, adipocytes and fibroblasts (Mafi et al. 2011; Gattegno-Ho et al. 2012). They can be harvested from a variety of sources, the most common of which are the bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) (Black et al. 2007; Gutierrez-Nibeyro 2011).

According to the systematic review of studies on human patients performed by Mafi et al (2011) BM-MSCs seem to have a higher differentiation potential than MSCs of other origin. However, there are certain benefits of choosing AD-MSCs over BM-MSCs. The relative ease and repeatable access to subcutaneous tissue together with the simple isolation procedure are some of these (Black et al. 2007; Punwar & Khan 2011). Also, harvesting of the bone marrow is painful and includes potential risks (Punwar & Khan 2011). In dogs bone marrow aspirates are most commonly obtained from the proximal humerus, proximal femur or tuber coxae (Fortier & Travis 2011). Adipose tissue in dogs has been collected from the abdominal, thoracic and inguinal areas ad from the region of the falciform ligament (Black et al. 2008).

The mechanism of action of MSC therapy is not completely understood, but the researchers believe that there are likely to be multiple receptors or pathways involved (Black et al. 2007). Currently it is not clear whether MSCs can differentiate into tissue-specific cells or is their potential therapeutic effect mediated through the secretion of immunomodulatory and trophic factors such as the cytokines and growth factors that affect the surrounding cells and alter the local inflammatory responses (Black et al. 2008; Fortier & Travis 2011; Fortier & Tuan 2012). MSCs might also recruit other endogenous cells to the site of the lesion (Black et al. 2007).

The isolation process of AD-MSCs includes mincing and washing, followed by collagenase digestion and centrifugation (Black et al. 2007). The pellet formed after the centrifugation, known as the stromal vascular fraction (SVF), contains in addition to AD-MSCs a diverse group of other cells such as fibroblasts, pericytes, circulating blood cells and endothelial cells (Black et al. 2007; Black et al 2008). This stromal vascular fraction is added into a solution and this suspension is injected back into the patient (Davatchi etl al. 2011).

2.3.1.2. Scientific evidence and clinical use

- In veterinary medicine

The most common clinical application of stem cells in clinical veterinary medicine is the treatment of musculoskeletal problems in horses and dogs (Fortier & Travis 2011). Autologous MSC therapy, meaning the use of the patient's own MSCs as a source, is already available for veterinary patients in certain countries (Black et al. 2007; Black et al. 2008). However, as the use of stem cells in veterinary patients is not closely supervised by any organization due to their so-called minimally manipulated nature, some researchers suggest that there might be clinical use of certain therapies with no shown efficacy in preclinical animal studies or *in vitro* (Fortier & Travis 2011).

Early results suggest that AD-MSC therapy might be useful as a treatment adjunct in the management of canine osteoarthritis. The only randomized, blinded, placebo-controlled clinical trial investigating the effect of stem cell therapy on canine hip osteoarthritis was performed by Black et al (2007). In this study 18 dogs with bilateral coxofemoral joint osteoarthritis received intra-articularly either 4,2-5 million viable stem cells prepared from the dog's own fat tissue or a similar volume of phosphate buffered saline. Statistically significant improvements in lameness and in pain and in the range of motion of the treated joint were seen in the treatment group compared both to the control group and to the baseline. However, no objective outcome variables were used in this study.

Black et al (2008) performed also a pilot study to evaluate the clinical effect of a single intra-articular injection of AD-MSCs in 14 dogs with osteoarthritis of the elbow joint. In this study a statistically significant improvement from baseline was demonstrated in the orthopedic examination scores and owner scores. However, this study was not placebo-controlled.

MSC therapy has also been studied in horses. Frisbie et al (2009) studied the clinical, biochemical and histologic effects of intraarticularly administered adipose-derived stromal vascular fraction or bone marrow-derived MSCs in an equine model of osteoarthritis. Twenty-four horses were divided into three study groups of eight horses. The horses in the control group received placebo while the horses in the two study groups received either adipose-derived stromal vascular fraction or bone

marrow-derived mesenchymal stem cells into the middle carpal joint. Although the levels of prostaglandin E_2 decreased in the synovial fluid, no significant improvements were demonstrated in the clinical or histopathological examination.

Based on the unremarkable results of the aforementioned study and the lack of other studies proving MSCs to have any effect on osteoarthritis, MSC therapy is currently not generally recommended to be used as a part of osteoarthritis treatment in the equine practice (McIlwraith 2011).

- In human medicine

In human medicine MSCs have been shown to have potential in the treatment of musculoskeletal disorders such as osteonecrosis of the femoral head, osteogenesis imperfecta, degenerative disc disease and Duchenne muscular dystrophy (Mafi et al. 2011).

The effect of MSC therapy on osteoarthritis has also been studied in humans. Davatchi et al (2011) examined the use of bone marrow-derived MCSs on four human patients with knee osteoarthritis. There were modest improvements in the pain scores, in physical examination and in the range of motion of the treated joint.

The study performed by Emadedin et al. (2012) evaluated the effects of intra-articular injection of bone marrow-derived autologous mesenchymal stem cells in six patients requiring joint replacement surgery due to knee osteoarthritis. The main objective of the study was to evaluate the safety of the treatment. Other parameters such as pain, functional status of the knee and walking distance were also evaluated and were shown to improve up to six months after the injection. An increase in the cartilage thickness, extension of the repair tissue over the subchondral bone and a decrease in the size of edematous subchondral patches was noted in three of the patients. No adverse effects were noted.

In human medicine the MSC therapy is currently not a part of the routine treatment regimen of patients with osteoarthritis. Before the wide clinical use of MSCs can be recommended, further studies are needed, as little is currently known of the cellular characteristics and endogenous functions of MSCs. (Mafi et al. 2011.)

2.3.2. Autologous conditioned serum

2.3.2.1. Mechanism of action

Catabolic cytokine interleukin 1 (IL-1) is the most potent known mediator of cartilage breakdown and it is a major inflammatory mediator in joint diseases (Frisbie et al. 2007; Baltzer et al. 2009). Interleukin 1 receptor antagonist (IL-1Ra) prevents the interaction of IL-1 with the cell surface receptors thus blocking the inflammatory cascade initiated by IL-1 (Baltzer et al. 2009; Chevalier & Kemta-Lepka 2010; Textor et al. 2011). However, there are thousands of IL-1 receptors on the surface of every fibroblast and yet bonding of only a few of them is enough to induce the cellular responses to IL-1 (Textor 2011). Therefore, to prevent the effect of IL-1, the ratio of IL-1Ra to IL-1 must be quite high (Textor 2011).

Autologous conditioned serum is one of the new biological therapies that have emerged as potential treatment options for human and equine osteoarthritis (Hraha et al. 2011). This therapy was developed in the mid-1990s in an attempt to create an injectable material that would be rich in endogenous IL-1Ra (Baltzer et al. 2009). ACS is considered as the endogenous source of IL-1Ra, since the major natural source of IL-1Ra are the blood monocytes (Evans 2005; Fox & Stephens 2010). Different methods to stimulate their IL-1Ra-production have been developed (Evans 2005).

Most commonly ACS is produced by incubating venous blood with medical grade class beads which induces the peripheral blood leukocytes to produce elevated amounts of endogenous anti-inflammatory cytokines such as IL-1Ra, IL-4, IL-10 and growth factors such as fibroblastic growth factor-1, hepatocyte growth factor and transforming growth factor-β1 (Frisbie et al. 2007; Rutgers et al. 2010). These anti-inflammatory cytokines and growth factors accumulate in the serum which is then harvested as the therapeutic agent (Wehling et al. 2007; Rutgers et al. 2010; Hraha et al. 2011). After centrifugation and extraction, ACS can be either stored for later use or injected into the lesion site (Wehling et al. 2007).

Despite the already ongoing clinical use, the exact mechanism of action of ACS in the osteoarthritic joint is not yet fully understood (Rutgers et al. 2010). Even the data available on the actual composition of the conditioned serum is limited (Rutgers et al. 2010). Because the stimulation increases the concentration of IL-1Ra to concentrations of as much as 140-fold greater than other anti-inflammatory proteins found in ACS, IL-

1Ra is assumed to be one of the major mediators behind the clinical improvement in patients with osteoarthritis (Frisbie et al. 2007; Hraha et al. 2011).

However, ACS is not purified IL-1Ra, as it also contains many blood-derived substances and its effects are stronger and longer-lasting than what researchers think could be expected from a short series of injections containing merely IL-1Ra (Evans 2005; Textor 2011). Thus some researchers suggest that the clinical effects are caused by the synergistic effect of all factors in the ACS (Wehling et al. 2007).

As the efficacy of ACS is currently based merely upon the improvement in clinical signs and symptoms, the possible disease-modifying effects that could occur in response to the presence of growth factors are yet to be determined. (Evans 2005.)

2.3.2.2. Scientific evidence and clinical use

- In veterinary medicine

Commercial ACS therapies are currently not available for dogs, but for horses ACS therapy, also known as the IL-1Ra protein (IRAP) therapy, is available also in Finland (Wehling et al. 2007; Fox & Stephens 2010). In horses, the most common clinical application of ACS is the intra-articular treatment of osteoarthritis (Textor 2011; Baltzer et al. 2009). In horses it has also been used in some cases prophylactically after arthroscopy for its potential anti-inflammatory and chondroprotective effects (Textor 2011).

Studies on dogs are currently lacking, but the effects of ACS therapy have been studied on equine models of osteoarthritis. Frisbie et al (2007) evaluated the clinical, biochemical and histologic effects of intra-articularly administered ACS compared to a placebo in an equine model of osteoarthritis. Osteoarthritis was induced in the middle carpal joint of all horses. In the 8 horses that were treated once a week for four weeks with ACS starting 14 days after the induction of osteoarthritis, significant clinical improvement in lameness and also significantly decreased synovial membrane hyperplasia were demonstrated. Such changes were not seen in the eight placebo-treated horses.

- In human medicine

In humans ACS is marketed as Orthokine (Orthogen AG, Dusseldorf, Germany) that has been available for clinical use since 1998 in several European countries (Baltzer et al. 2009; Fox & Stephens 2010). In human medicine it is used in the treatment of osteoarthritis, lumbar pain of neurogenic origin and muscle injuries (Wehling et al. 2007; Textor 2011).

In the Orthokine method approximately 50-60 ml of peripheral blood is drawn into the special syringes containing medical-grade class beads and is incubated for 24 hours (Fox & Stephens 2010; Evans 2005). After the separation from the blood cells, the conditioned serum is collected by centrifugation and filtering (Evans 2005). This serum is then returned to the patient in a series of up to six intra-articular injections given at weekly intervals (Evans 2005).

The effects of ACS therapy have been studied on humans. The randomized, placebo-controlled double-blind clinical trial by Baltzer et al (2009) compared the effects of intra-articularly administered ACS to hyaluronan and saline in 376 human patients with knee osteoarthritis. A reduction in pain was noted in all study groups, but the effects of ACS were superior to those of hyaluronic acid and saline. No differences between the effects of hyaluronic acid and saline were demonstrated. In addition to this, the therapeutic effect seemed to be rather long-lasting: there were still statistically significant differences between the ACS group and the two other study groups at a follow-up performed 2 years after the initial study. No serious side-effects of ACS administration were noted during the observation period.

Although ACS therapy seems generally a safe and treatment option, further studies are required to confirm its efficacy in the treatment of osteoarthritis (Wehling et al. 2007; Fox & Stephens 2010).

2.3.3. Platelet-rich plasma

2.3.3.1. Mechanism of action

As the first cell type to arrive at the site of injury platelets take part in the early phases of inflammation and in maintaining the healing process (Cole et al. 2010; Kon et al.

2011). Originally platelets were thought to act merely in the clotting process, but they also contain growth factors and cytokines that are crucial in the soft tissue healing and bone mineralization (Sampson et al. 2010). Many of the bioactive proteins released by platelets have been shown to attract the macrophages, mesenchymal stem cells and osteoblasts that play a role in tissue regeneration and healing (Sampson et al. 2010).

The use of autologous growth factors especially in the treatment of musculoskeletal injuries has recently received plenty of attention (Kon et al. 2010; Kisiday et al. 2012). There is scientific interest in the effects of the growth factors especially on osteoarthritis and cartilage repair because of their ability to recruit chondrogenic cells, stimulate proliferation and enhance synthesis of cartilage matrix (Kon et al. 2010; Spakova et al. 2012).

Growth factors stored in the platelets such as platelet-derived growth factor (PDGF), transforming growth factor β1 (TGF-β1), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), basic fibroplastic growth factor (bFGF) and epidermal growth factor (EGF), have been shown to take part in the regulation and synthesis of the articular cartilage (Cole et al. 2010; Kon et al. 2011a ; Filardo et al. 2012). Platelets are also a source of cytokines, chemokines and other proteins that take part in the stimulating of chemotaxis, cell proliferation and maturation, modulation of inflammatory molecules and the attraction of leukocytes (Kon et al. 2011a). Other substances stored in the platelets such as metalloproteinases, antibacterial and fungicidal proteins, coagulation factors, calcium ions, serotonin and dopamine can also have an effect on the inflammation and tissue regeneration (Cole et al. 2010; Kon et al. 2011a; Filardo et al. 2012).

It has been thought, that the delivery of high concentrations of platelets would lead to the delivery of high concentrations of the aforementioned growth factors as well and thus have the potential to augment or stimulate the same healing process that normally occurs after injury (Sampson et al. 2010; Stief et al. 2011; Spakova et al. 2012). Intra-articular administration of a platelet-rich concentrate has also been considered to have potential to slow down the progression of osteoarthritis by stimulating the cartilage anabolism (Stief et al. 2011).

Of the platelet-derived products available, platelet-rich plasma (PRP) is among the most commonly used (Textor 2011; Kisiday et al. 2012). As the name implies, platelet-rich plasma is defined as an autologous concentration of platelets in a small plasma

volume (Spakova et al. 2012). Other options such as platelet-rich fibrin clot (PRFC), platelet-rich clot releasate and platelet concentrate, are also available (Textor 2011). In addition to the growth factors, PRP also contains plasma proteins such as fibrin, fibronectin and vitronectin that act as mesenchymal cell adhesion molecules (Spakova et al. 2012).

Following the injection into the damaged tissue, the platelets of PRP begin active secretion of growth factors within 10 minutes with more than 95 % of the total amount released within the first hour (Kon et al. 2011a). Although the secretion of growth factor occurs mainly in the first hour of the injection, the platelets remain viable for 7 days and continue to release growth factors. Thus a single injection into the damaged tissue might be sufficient at least in most cases (Kon et al, 2011a; Leong et al 2012).

PRP is derived from the anticoagulated autologous blood by centrifugation that eliminates most of the red blood cells and concentrate platelets based on the specific gravities of each cell type (Foster et al. 2009; Kon et al. 2011b; Kisiday et al. 2012). The achieved platelet concentration varies, but is generally at least 4-5 or even over 10 times higher than that of the whole blood (Kon et al. 2011b; Kisiday et al. 2012). The platelet concentrate is activated using calcium chloride resulting in the formation of platelet gel that can confine the secretion of growth factors to the chosen site (Kon et al. 2011b).

PRP can be prepared using standard blood tubes and laboratory centrifuges, but there are also many different commercial systems available for PRP production. Some of the advantages of these systems are the ease of use, rapid PRP production and maintenance of sterility. For horses there is even a stall-side preparation system available (E-PET, Pall Animal Health, Port Washington, NY, USA). (Textor 2011.)

The three main commercial methods of producing PRP are selective blood filtration, single-spinning methods and double-spinning procedures of which the two latter are the most common ones in the clinical use (Filardo et al. 2012). The systems differ in the initial blood volume withdrawn, in the final volume of PRP and in the final platelet concentration (Leong et al. 2012). There are also differences in the speed and number of centrifugations, in the use of anticoagulant, in the presence of other cells such as leukocytes in the preparation, in the use of activators and in the storage modalities (Filardo et al. 2012; Leong et al. 2012). These differences in preparation and

administration make it difficult to compare the results of different studies on PRP challenging both in human and veterinary medicine (Textor 2011; Kon et al. 2011a).

2.3.3.2. Scientific evidence and clinical use

- In veterinary medicine

Platelet-rich concentrates (PRC), such as PRP have gradually started to gain attention in the treatment of various acute and chronic sport injuries, particularly tendon and ligament injuries, in both human and veterinary medicine (Stief et al. 2011; Textor et al. 2011). Although PRP therapy is also used to treat joint diseases in horses, there are currently no clinical studies on the effects of PRP therapy in the management of canine or equine osteoarthritis (Textor et al. 2011).

However, Milano et al (2010) studied the effects of PRP in the treatment of full-thickness articular cartilage lesions of the stifle joint in an experimental ovine model. The ten sheep in the treatment group received PRP either in liquid form or together with fibrin glue 12 months after the procedure. On both macroscopic and histological evaluation the amount of repair tissue was the highest in the PRP treated joints compared to the five untreated animals, which might indicate that PRP has a positive effect on cartilage repair.

In horses PRP therapy is mostly used in the treatment of tendon and ligament injuries, in some cases in combination with the stem cells. As PRP is intended to support and enhance tissue healing as an anabolic agent, in horses it is recommended to be used after an acute traumatic injury to musculoskeletal tissues. (Textor 2011.)

- In human medicine

In human medicine autologous PRP was first used in 1987 by Ferrari et al after open heart surgery. The first clinical study on the use of PRP to supplement cancellous bone graft in humans was made in 1998 with significant improvements detected in both radiologic and histologic scores of bone density. (Textor 2011; Frizziero et al. 2012.)

The use of PRP therapy is more established in human medicine compared to veterinary medicine as it is used in a wide variety of different fields such as sports

medicine, orthopaedics, dentistry, dermatology, ophthalmology and plastic and maxillofacial surgery (Cole et al. 2010; Kon et al. 2011a). The use of PRP in cartilage repair is actually relatively new (Frizziero et al. 2012).

Despite the fact that the use of PRP in the treatment of tendon, ligament and bone injuries is being extensively studied there are currently only few studies evaluating the effects of PRP on articular cartilage. The dose of platelets that best stimulates cartilage is also yet to be determined. (Kisiday et al. 2012.)

Currently available clinical studies on humans support the role of PRP in the treatment of cartilage lesions (Filardo et al. 2012). In the study performed by Kon et al (2010) investigating the effect of intra-articular PRP on human knee osteoarthritis statistically significant improvements were demonstrated both in pain and function compared to baseline. However, there was no control group in this study. Similar results were demonstrated in a study performed by Sampson et al (2010) evaluating the effects of three intra-articular PRP injections at 4 week intervals in 14 human patients with primary and secondary osteoarthritis.

PRP might even be a challenger to hyaluronic acid as indicated by results from the study by Spakova et al (2012). In their study the effects of intra-articular PRP were compared to those of intra-articular hyaluronic acid in 120 human patients with knee osteoarthritis. The injections were given once a week on three consecutive weeks. A statistically significantly better improvement in the clinical signs was noted on the evaluation three and six months after the last treatment in the group that received PRP compared to the group that received hyaluronic acid. PRP was compared to hyaluronic acid also in the study by Kon et al (2011b). In this study a longer-lasting and greater improvement in pain and function was seen when using PRP than when using hyaluronic acid.

The absence of significant adverse effects, immune reactions and disease transmission are some of the advantages of the use of autologous PRP (Cole et al. 2010; Filardo et al. 2012; Frizziero et al. 2012). However, there is currently no data available on the possible long-term adverse effects (Frizziero et al. 2012). Many of the studies also lack controls, have small sample sizes and do not define a standardized PRP preparation which makes interpreting and comparing the study results rather challenging (Foster et al. 2009; Kon et al 2011a). Further research on factors such as the harvesting methods, the ideal concentration of platelets, the amount of leukocytes

and growth factors in the preparations and the protocols of delivery is also needed (Cole et al. 2010; Leong et al. 2012).

Although in humans the concurrent NSAID therapy is avoided based on its potential inhibitory effects on platelets, some authors find this to be an unnecessary precaution since the platelets are delivered in an already activated state (Textor 2011).

2.4. BOTULINUM NEUROTOXIN TYPE A

2.4.1. Mechanism of action

There are seven serotypes (A-G) of toxins produced by the neurotoxigenic strains of *Clostridium* bacteria. Of these botulinum neurotoxin A (BoNT-A) is the most common serotype in clinical use. (Borodic et al. 2001.)

The analgesic effect of intra-muscularly administered BoNT-A is attributed to a reduction in muscle activity caused by the inhibition of acetylcholine release at the neuromuscular junction and following local muscle paralysis (Borodic et al. 2001). However, BoNT-A seems also have effects beyond the neuromuscular junction, which is a phenomenon that was noticed when the analgesic effect of BoNT-A injection was found to occur earlier and to a greater degree than the decreased muscle tone (Aoki 2003; Singh 2010). Persistent joint pain can lead to sensitization of articular nociceptors and release of neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) (Schaible 2006; Birklein & Schmelz 2008; Innes 2012). In studies performed on animal models of osteoarthritis BoNT-A has been shown to inhibit the release of these substances (Rapp et al. 2006; Lucioni et al. 2008).

2.4.2. Scientific evidence and clinical use

- In veterinary medicine

Botulinum toxin is currently not in clinical use in veterinary medicine, but there are studies on its use on animal models. The effect of BoNT-A on canine osteoarthritis has been studied in a small pilot study by Hadley et al. (2010). They investigated the effect of intra-articularly administered botulinum toxin in five dogs with unilateral chronic

moderate to severe osteoarthritis of either elbow or hip joint. Twenty five IU of BoNT-A, a dose extrapolated from human studies, of BoNT-A was injected into two elbow joints and three hip joints and the outcome was evaluated at 2, 4, 8 and 12 weeks after the injection by pressure platform gait analysis and by owner assessment. The study showed improved ground reaction forces in the treated limb and some improvement in the owner's assessment was reported in four out of five dogs.

No immediate adverse effects were noted in this study, but in two of the dogs there was mild redness and swelling at the injection site and increased lameness during the first 24-48 hours after the injection. However, no systemic adverse effects were noted and the local reactions subsided within two days.

Intra-articular BoNT-A has also been studied in horses. DePuy et al (2007) performed a pilot study on the effect of intra-articular BoNT-A on lameness associated with acute synovitis in an equine model. Four experimental horses were divided into pairs that were given either 50 units of BoNT-A or saline into the middle carpal joints. Acute synovitis was induced 14 days later with equine IL-1β injection and synovial fluid analysis, clinical evaluation of lameness and kinematic gait analysis were performed the next day. On histology and cytology synovitis was observed in all horses, but the other horse of the BoNT-A group showed no changes in the baseline gait analysis. The other three horses all developed prominent forelimb lameness. However, the interpretation of the results and determining whether or not BoNT-A prevented the lameness is rather challenging due to the small sample size. No detectable adverse effects caused by the BoNT-A injection were noted in this study either.

- In human medicine

The analgesic properties of intra-muscularly administered BoNT-A are well documented and in humans BoNT-A has been shown to be effective for treatment of painful movement disorders, spasticity, myofascial pain and conditions associated with increased muscle tone, abnormal posture and pain (Borodic et al 2001; Chou et al. 2010).

Also the effects of intra-articular BoNT-A have been studied in the treatment of chronic joint pain in humans. An example of these studies is the one performed by Singh et al. (2010) on the pain-relieving effect of intra-articular BoNT-A on chronic

knee pain after total knee arthroplasty. The patients received either 100 IU of BoNT-A or saline as a single intra-articular injection and the outcome was assessed during the follow-ups at two, three, four and six weeks after the injection. There was a statistically significant improvement in pain and function in the treatment group compared to the control group during the follow-up two months after the injection. Although the duration of the pain-relieving effect of BoNT-A injection was also shown to be longer than that of the placebo, the mean duration of the pain-relieving effect in the study group was only 39 days. Thus it was speculated, that a higher dose or more frequent administration might be needed for the clinical application of BoNT-A.

Mahowald et al (2009) performed two randomized controlled pilot studies to investigate the effect of intra-articularly administered BoNT-A on joint pain in patients with painful shoulder or knee joints. In both studies the decrease in pain severity produced by BoNT-A was significant while that caused by placebo injection was not.

The effect of intra-articular BoNT-A on chronic joint pain has also been investigated in smaller studies, some of which lacked the control group. Chou et al (2010) performed a study to evaluate the therapeutic effects of intra-articular BoNT-A in humans with advanced knee osteoarthritis. Patients were radiographically verified having either stage III or IV osteoarthritis according to the Kellgren-Lawrence classification. Clinically significant improvement in pain and stiffness were noted at 1 month after the first injection, but statistical significance was noted only at 3 months after the injection and only for the pain subscale in stage III osteoarthritis. No adverse effects, such as increases in joint inflammation, periarticular muscle weakness, fever or fatigue or changes in neurosensory testing of the lower extremities have been noted in the human studies performed this far (Mahowald et al. 2009; Singh et al. 2009a;Singh et al. 2009b; Chou et al. 2010).

2.5. OTHER OPTIONS

In search for the optimal treatment option for osteoarthritis, a myriad of other options for intra-articular therapy of osteoarthritis have been studied both in human patients and in animal models of osteoarthritis. Examples of these compounds that have been administered experimentally are orgotein, silicone, magnesium sulfate, chondroitin sulfate, calcitonin, saline solution, NSAIDs, somatostatin, chloroquine,

mucopolysaccharide polysulfuric acid ester, lactic acid solution, thiotepa cytostatica, polynucleotides and prolotherapy (Edwards 2011; Hameed & Ihm 2012). However, since the effects of many of these have been investigated only in small uncontrolled studies, no definitive recommendations can yet be given regarding their use (Hameed & Ihm 2012).

DISCUSSION

Intra-articular medications, especially corticosteroids, are used in the management of canine osteoarthritis, although the full extent of their use among veterinarians is not known. There are probably many reasons why the intra-articular medications are not as commonly used in dogs as they are in human medicine and in the equine practice. First of all, there is more scientific evidence on the efficacy of intra-articular medications in the treatment of osteoarthritis in human medicine as the amount of scientific publications on the matter is very scarce in dogs. Most of these studies are also performed on experimental canine models of osteoarthritis and have been focused on evaluating the effects of the medications on the disease progression rather than on the clinical signs such as pain or the degree of lameness.

Also, unlike in humans and in horses, arthrocentesis is not a routine procedure in dogs and most veterinary practitioners have not been trained to perform it. Arthrocentesis of the commonly affected joints, such as the hip and elbow joints, is most likely going to require some practice before the practitioner can perform it comfortably.

An interesting remark is that the mere removal of some of the synovial fluid is a short-term symptomatic treatment as such, as it helps to clear the joint from the altered inflammatory synovial fluid. Also, it has been speculated, if intra-articular injection of any agent, such as saline, could have short-term beneficial effects due to the dilution of the inflammatory cytokines and enzymes in the joint cavity. (Colen et al. 2012.)

As a conclusion of the studies, the practitioner, in my opinion, is not likely to do much harm by incorporating the intra-articular corticosteroids and hyaluronic acid in the treatment of an osteoarthritic patient in cases where other treatment options have been tried and proven inadequate and euthanasia is not yet an option. In these cases, however, strict aseptic technique must be followed, the patient has to be suitable for anaesthesia and the owner needs to be aware of the lack of the strong scientific proof of the efficacy of intra-articular medications in dogs.

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