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Effect of corticosteroids on articular cartilage after intra-articular injection as treatment of osteoarthritis

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*Uppsala
2017*

Veterinärprogrammet, examensarbete för kandidatexamen
2017:75

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Kortikosteroiders effekt på ledbrosk efter intraartikulär injektion som behandling av osteoartrit

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biomedicin och veterinär folkhälsovetenskap

Omfattning: 15 hp

Nivå och fördjupning: Grundnivå, G2E

Kurstitel: Självständigt arbete i veterinärmedicin

Kurskod: EX0700

Program/utbildning: Veterinärprogrammet

Utgivningsort: Uppsala

Utgivningsår: 2017

Serienamn: Veterinärprogrammet, examensarbete för kandidatexamen

Delnummer i serie: 2017:75

Elektronisk publicering: <http://stud.epsilon.slu.se>

Nyckelord: Osteoartrit, ledbrosk, kortison, betamethason, triamcinolon acetonid, methylprednisolon acetat

Key words: Osteoarthritis, articular cartilage, corticosteroids, betamethasone, triamcinolone acetonide, methylprednisolone acetate

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SAMMANFATTNING

Osteoartrit (OA) är ett stort problem hos hästar då det orsakar upp till 60% av alla hältor. OA är en inflammatorisk och progressiv sjukdom som uppkommer när ledens naturliga balans mellan anabola och katabola processer rubbas och jämnvikten förskjuts så att de katabola processerna tar över. Behandlas inte OA i tid kommer denna obalans att fortgå och efter en tid kommer degenerativa, kroniska skador på ledbrosket att uppstå. När sjukdomen har gått så långt är det svårt att behandla med lyckat resultat. Lyckas man upptäcka sjukdomen i ett tidigt stadium och sätter in behandling direkt är prognosen bättre.

Idag är intraartikulär injektion av kortikosteroider ett vanligt sätt att behandla OA. Trots att kortikosteroider är effektiva när det gäller att ta bort kliniska symptom så som hälta är denna behandlingsmetod något kontroversiell och omdiskuterad. Anledningen till detta är att vissa hävdar att kortikosteroider har negativa effekter på ledbrosket.

Inom veterinärmedicin är det framförallt tre olika kortikosteroider som är populära för intraartikulär behandling i samband med OA hos häst, *betamethason*, *triamcinolon acetonid (TA)* och *methylprednisolon acetat (MPA)*. Syftet med denna uppsats är att jämföra dessa tre farmakologiska formuleringar för att utvärdera om det finns några uppenbara skillnader mellan dem, speciellt i avseende på hur ledbroskets matrix molekyler påverkas och hur effektiva de är på att reducera hälta.

Det är svårt att utifrån de studier som idag finns tillgängliga att dra någon konkret slutsats om vilket av de tre kortisonpreparat som är det bästa alternativet för behandling av OA. Anledningen till detta är att studierna som är gjorda är utformade på olika sätt och det är därför svårt att jämföra dem med varandra. Det som kan konstateras är dock att det inte finns några uppenbara tecken på att intraartikulär behandling med *TA* eller *betamethason* leder till skador på ledbrosket så länge man använder korrekta doser och låter hästen vila under den tid som kortisonet verkar. *MPA* är mer tveksamt, här har många studier visat på negativa effekter hos ledbrosket. Dock har man i dessa studier använt friska hästar, höga doser och lederna har injicerats med täta intervall. Det skulle vara intressant att se om man kunde se samma negativa effekter på ledbrosket även vid lägre doser och mindre frekventa injektioner av *MPA*. Sammanfattningsvis kan det konstateras att studier där dessa tre typer av kortikosteroider jämförs på likvärdigt sätt krävs för att kunna dra bättre slutsatser om vilket typ som fungerar bäst.

SUMMARY

Osteoarthritis is a huge problem in equine veterinary medicine since it causes up to 60% of all lameness. OA is an inflammatory and progressive disease, which occur when the natural balance between anabolic and catabolic processes is disturbed. If this imbalance continues and no treatment is insert, degenerative and chronic damage on the articular cartilage will occur. When the disease comes to this irreversible stage it is difficult to achieve a successful treatment but in an early stage of the disease treatment may improve the prognosis.

Today intra-articular injections with corticosteroids is a common treatment of OA. Corticosteroids are effective to reduce lameness but the treatment is controversial and heavily discussed. The reason for this is that some people believe that corticosteroids affect the articular cartilage metabolism negatively.

In equine veterinary medicine, mainly three types of corticosteroids are used, *betamethasone*, *triamcinolone acetonide (TA)* and *methylprednisolone acetate (MPA)*. The purpose of this study is to investigate if there are any differences between these three pharmaceutical preparations, with focus on the effect on articular cartilage matrix molecules and how effective their therapy reduces lameness.

Since studies within the subject are designed in different ways it is difficult to compare them in a fair way. Therefore, it is impossible to determine which of the three corticosteroids that works in the most favorable way in reducing OA. What can be said is that there is no significant evidence that *TA* or *betamethasone* would be harmful to the articular cartilage as long as the dosage is correct and the horse rest while the drug is acting. *MPA* is a bit more doubtful. Studies have shown that *MPA* affect the articular cartilage in a negative way, but the methods in these studies can be questioned since the horses are injected with high doses and frequent intervals. Studies with more relevant doses and injection intervals would be wishful. As a conclusion, more studies which compare these three types of corticosteroids are necessary to be able to say which is the best alternative as treatment of OA in horses.

INTRODUCTION

Osteoarthritis (OA) is a huge problem in equine veterinary medicine since it causes up to 60% of all lameness (McIlwraith *et al.*, 2012). In a healthy joint there is a balance between catabolic and anabolic processes and when this balance is disrupted OA can evolve (Frisbie, 2005). OA is a low-grade inflammatory and progressive joint disease that include all structures in the synovial joint such as joint capsule, synovial fluid, articular cartilage, subchondral bone, tendons and ligaments. Damage to any of these structures leads to biochemical imbalance and with time, degradation of articular cartilage will occur (Heinegård *et al* 2011).

Today there are no available methods for diagnosing OA at an early stage of the disease. In order to determine OA the horses must show clinical signs such as lameness or swelling of the joint. In this stage the inflammation is well developed and potent anti-inflammatory drugs are required to take the horse back to training. Corticosteroids are a common way to treat horses diagnosed with OA(Frisbie, 2005). Corticosteroids relieve the symptoms of the disease but it is well discussed how these drugs negatively affects the articular cartilage (McIlwraith *et al.*, 2012). In equine sports medicine mainly three types of corticosteroids are used for intra-articular treatment of OA. These are *betamethasone*, *triamcinolone acetonide*, *betamethasone esters* and *methylprednisolone acetate* (McIlwraith *et al.*, 2012).

This essay will describe the anatomy and physiology of the synovial joint, the pathogenesis of OA and then discuss benefits compared with the side effects of corticosteroids as treatment of OA. Is there a difference between short-acting and long-acting corticosteroids with respect to positive clinical effect and negative side effects on the articular cartilage?

MATERIAL AND METHODS

This study is based on scientific papers, studies and publications relevant to the topic. The following web site was used for finding materials:

<http://www.pubmed.com>

The phrases used for search were “corticosteroids”, “articular cartilage”, “triamcinolone acetonide”, “betamethasone esters”, “methylprednisolone acetate”.

These phrases were combined with “equine AND + OR horse”.

Basic anatomy, physiology and pathology about synovial joints is taken from the book *Joint Disease in the Horse*.

LITTERATURE REVIEW

Synovial joint – anatomy and physiology

The synovial joint has two major functions, enable movement and transfer load. To enable these functions the joint consists of several structural components such as hyaline cartilage, subchondral bone, joint capsule with synovial fluid, tendons and ligaments. The synovial joint is defined as an organ where all structures interact with each other to promote optimal function of joint movement (McIlwraith *et al.*, 2015).

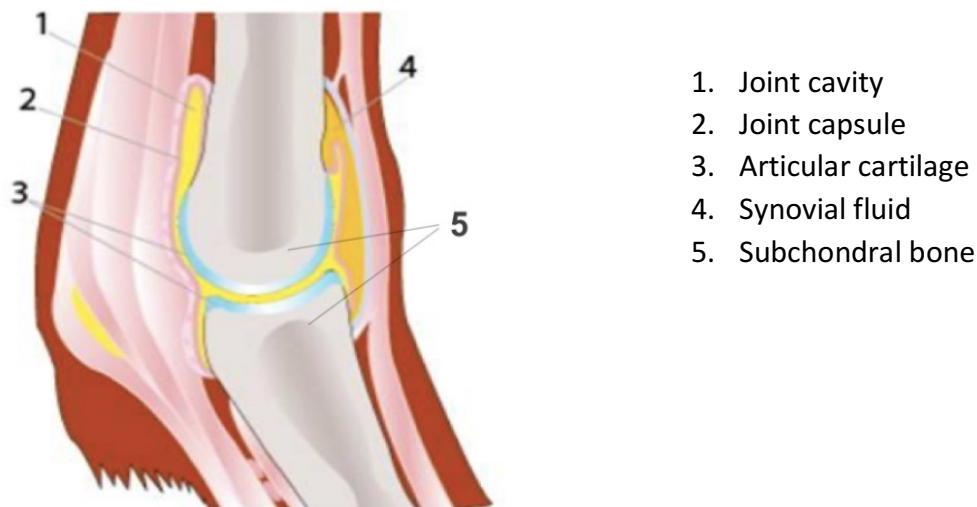


Figure 1. *General anatomy of a synovial joint (Gessbo 2004)*

Joint capsule

The capsule is comprised of two layer, the outer fibrous layer, called stratum fibrosum and the inner layer, called the synovial membrane. The fibrinous layer is interacting with extra-articular structures such as ligaments and tendons and is important for the stabilization of the joint. The inner synovial membrane can be further divided into two layers, the inner synoviocytes layer (*intima synovialis*) and the subsynovial layer (*stratum subsynoviale*). Two types of synoviocytes are present in the intima synovialis, these are type-A and type-B synoviocytes. Type-A are responsible for phagocytosis whereas type-B produce and secrete proteins (McIlwraith *et al.*, 2015).

Synovial fluid

The synovial fluid is an ultra-filtrate of blood plasma but it also contains components produced by the synovial membrane lining cells, for example hyaluronan (HA) and lubricin (Köning *et al* 2009). HA gives the synovial fluid its viscosity. The viscous nature of the synovial fluid allows it to support transient shear stresses and to absorb some of the energy generated by movement. The levels of HA are reduced in synovial fluid during development of OA (Sofat, 2009). Also reduced chain length of HA can be seen in injured joints (Brown *et al.*, 2007). The

glycoprotein lubricin and hyaluronan together acts synergistically to lubricate the surface and articular cartilage (Bonnievie *et al.*, 2016). Lubricin adheres to the surface of the articular cartilage and synovial membrane and reduce the surface tension and forcing opposing articular surfaces apart to prevent fusion (McIlwraith *et al.*, 2015).

Articular cartilage

Articular cartilage is a highly specialized connective tissue that covers the surface of the subchondral bone (McIlwraith *et al.*, 2015). It is avascular and rich in extracellular matrix, which consists of collagen, proteoglycans, non-collagenous proteins and water (Van Weeren *et al.*, 2016).

Chondrocytes

Chondrocytes are the only type of cells in adult cartilage and the cell density is low. The chondrocytes are responsible for synthesis and organization of the extracellular matrix. Based on the organization of the chondrocytes, the articular cartilage can be divided into four zones. Zone I-III are the unmineralized zones and these are delineated from zone IV which is the calcified cartilage by the tidemark. Tidemark is the junction between calcified and non-calcified cartilage. In zone I (*superficial zone*) the chondrocytes are relatively small and elongated and this zone has the highest cell density. Zone II (*transitional zone*) has larger and rounded cells and in zone III the chondrocytes are even larger and arranged in vertical columns (McIlwraith *et al.*, 2015).

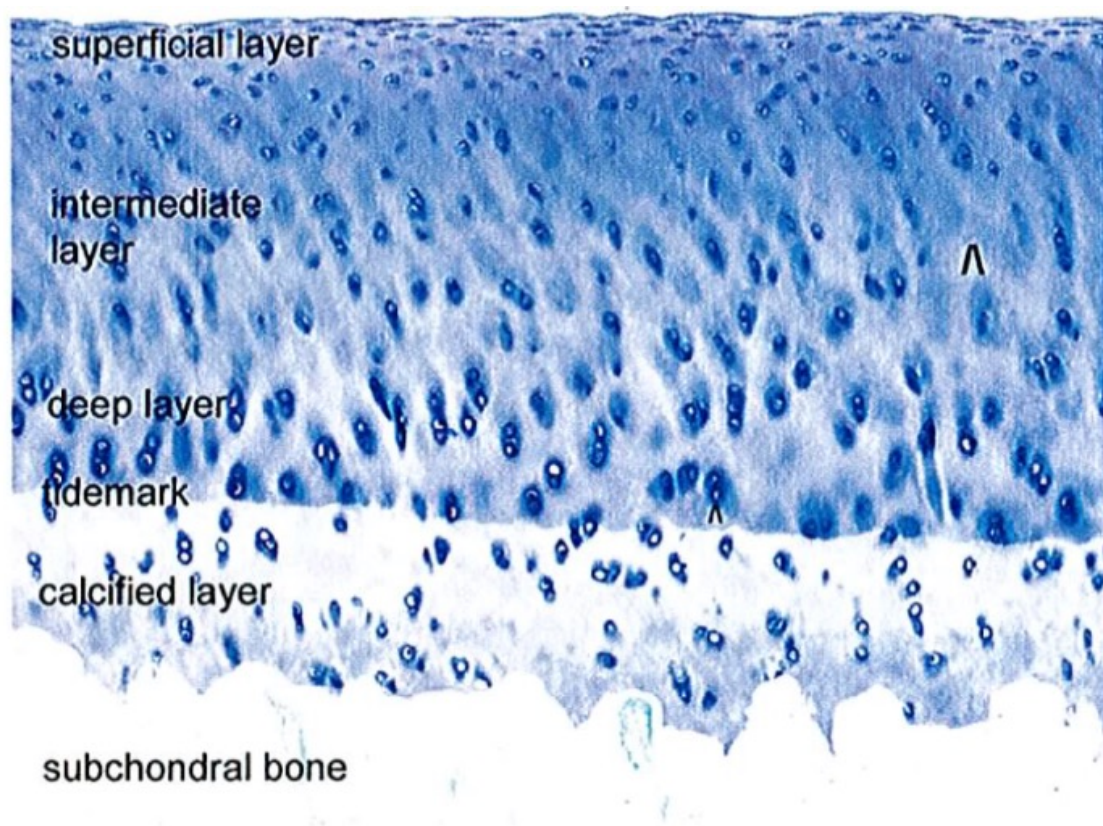


Figure 2. Immunohistochemistry section of Toluidine blue stained equine articular cartilage from the third carpal bone of a four-year-old trotter (Skiöldebrand 2004)

Extracellular matrix

Extracellular matrix consists of collagen, proteoglycans, non-collagenous proteins, water and lipids. Most of the collagen (85-90%) is type II collagen. Type II collagen tensile strength to the cartilage and it has a higher hydroxylation of lysin and more glycosylation than other types of collagen. Type II collagen fibrils consists of three α chains that are wound around each other to form a triple helix. By cross-linking to each other the collagen fibrils form a three-dimensional meshwork. The inter fractions in collagen have a long half-life ($t^{1/2} = 300$ days) (Vandeweerde *et al.*, 2015). In the collagen framework proteoglycans are enmeshed. Proteoglycan consists of a protein core which one or more glucosaminoglycans are bound covalently to. Unlike collagen proteoglycans have a short half-life ($t/2 = 8$ to 10 days) (Vandeweerde *et al.*, 2015). The most common proteoglycan in adult cartilage is *aggrecan*. To the central protein core of aggrecan a large number of glucosaminoglycans, especially chondroitin sulphate, but also keratin sulphate are covalently attached (Platt *et al.*, 1998). Aggrecan also has three globular domain, G1, G2 and G3. G1 and G2 are placed at the N-terminal, which is the hyaluronan binding region. G3 is found at the C-terminal in the other end of the molecule. Between G2 and G3 most of the glucosaminoglycans are found. Glucosaminoglycans are negatively charged and therefore attracts water, which contributes to the elasticity of the cartilage. Loss of glucosaminoglycans therefore makes the cartilage less elastic and more sensitive for pressure. Another important molecule in extracellular matrix is Cartilage oligomeric matrix protein (COMP), which is a pentameric, non-collagenous glycoprotein. COMP binds to collagen I, II and IX. These bonds are essential for the three dimensional structure of the collagen network (Skiöldebrand, 2010).

Subchondral bone

The subchondral bone provides structural support to the overlying cartilage. The metabolism in the bone is high and it is well vascularized and innervated by nerves. The bone adapts to the load it is exposed to and during repeated high load thickness and subchondral bone sclerosis can be detected at the X-ray. Bone is composed of an inorganic component of mineral salts and an organic component of mainly type I collagen, proteoglycan, glucosaminoglycans and water. The inorganic phase gives the bone its hardness and rigidity whereas the organic phase provides flexibility and resiliency (McIlwraith *et al.*, 2015).

Tendons and ligament

Tendons and ligament are soft tissue which together with muscles support the joint mechanically and act cushioning. The ligaments can be extra-articular, called collateral ligaments or intra-articular (McIlwraith *et al.*, 2015).

Osteoarthritis

Osteoarthritis (OA) is a chronic, debilitating, low-grade inflammatory joint disease and one of the most common causes of lameness in horses. The risk factors for development of OA has been classified into two main groups, abnormal loading on normal cartilage (1) or normal loading on abnormal cartilage (2) (McIlwraith *et al.*, 2012).

In a healthy joint an equilibrium between anabolic and catabolic factors exists. During development of OA this balance is disturbed and the catabolic processes takes over the anabolic (Mueller *et al.*, 2011). OA can be initiated in different parts of the synovial joint, for example in synovial membrane. This is called a synovitis and initiates release of proinflammatory cytokines like IL-1 β and TNF- α . Increased levels of IL-1 β and TNF- α creates a cascade reaction leading to increased level of matrix-degrading enzymes such as matrix metalloproteinase (MMP), aggrecanases and prostaglandins. Also a reduction of extracellular matrix protein synthesis can be seen. It is not possible to diagnose OA at an early stage and when these catabolic processes continues it will end up with destruction and loss of articular cartilage. Early changes in articular cartilage are characterized by loss of proteoglycans and changes in the organization of collagen in the superficial zone. (Saarakkala *et al.*, 2010) When degradation continues and the disease progress to a later stage also a loss of collagen can be seen (Huenbner *et al.*, 2009).

Interleukin-1 β (IL-1 β)

IL-1 β is a pro-inflammatory cytokine which is important in the early development of OA. Increased levels of IL-1 β is found in synovial fluid in joints in which development of OA occurs. IL- 1 β binds to IL-1 receptors on the cell membrane and activate matrix metalloproteinases, aggrecanase and prostaglandin E2. There is also an increased number of IL- 1 receptors in osteoarthritic cartilage which also leads to increased sensitivity of IL- 1 β (Mueller *et al.*, 2011).

Tumor necrosis factor - α (TNF- α)

TNF- α is also a pro-inflammatory cytokine which acts synergistically with IL- 1 β . It stimulates expression of matrix-degrading enzymes and reduces synthesis of extracellular matrix. Like IL- 1 β , TNF- α inhibit chondrocytes to synthesis proteoglycans and collagen. (Mueller *et al.*, 2011).

Matrix metalloproteinase (MMP)

MMPs are zinc-dependent enzymes, synthesized by chondrocytes involved in extracellular matrix degradation. During development of OA when the levels of IL-1 β and TNF- α increase, these molecules binds to their respective cell receptor in chondrocytes and signaling pathways that result in upregulation of MMP. MMP-13 has proven to be especially important since it both can cleave collagen type II and proteoglycans in articular cartilage. It has been seen that levels of MMP-13 increase when chondrocytes are exposed to mechanical stress (Kawaguchi 2008).

Aggrecanases

Aggrecanases are extracellular proteases and belongs to a family called the ADAMTS. When talking about OA ADAMTS-4 and ADAMTS-5 are especially important (Abramson *et al.*, 2009). Increased levels of IL-1 β and TNF- α leads to increased expression of aggrecanases and this results in loss of aggrecan in the cartilage (Mueller *et al.*, 2011).

Prostaglandin E2 (PGE2)

PGE2 is an important inflammatory mediator and the production requires two enzymes, prostaglandin E synthase and cyclooxygenase 2 (COX-2). These enzymes are both induced by IL-1 β and because of this PGE2 levels increase as a result of higher concentration of IL-1 β (Mueller *et al.*, 2011). PGE2 inhibit synthesis of proteoglycans and increase the levels of ADAMTS-5 and MMP-13 which leads to degradation of aggrecan and collagen II (Attur *et al.*, 2008).

Nitric oxide (NO)

NO is produced by osteoarthritic chondrocytes and drives the catabolic process in the joint and is involved in the development of OA in many ways. IL-1 β stimulates overexpression of inducible nitric oxide synthase (iNOS) which leads to high levels of NO. NO has the properties to inhibit synthesis of proteoglycan and collagen in chondrocytes and also induce chondrocyte apoptosis (Mueller *et al* 2011).

CORTICOSTEROIDS

The optimal treatment of OA would be an agent that both relieve the symptoms of lameness (symptom-modifying OA drug, *SMOAD*) and produce disease modifying effects, (disease-modifying OA drug, *DMOAD*) by inhibit the progression (McIlwraith *et al.*, 2012). Corticosteroids are potent anti-inflammatory drugs and in that way helpful in reliving symptoms of OA but it has also been shown that corticosteroids negatively affect the cartilage, for example it decreases collagen type II synthesis and proteoglycan content (Todhunter *et al.*, 1998). Intra-articular injections of corticosteroids are therefore controversial and benefits compared with the side effects are heavily debated.

In equine veterinary medicine three corticosteroids are mainly used as treatment of OA, these are *betamethasone*, *triamcinolone acetonide (TA)* and *methylprednisolone acetate (MPA)* (McIlwraith *et al.*, 2012). Below these three pharmaceutical preparations will be compared to each other, especially with respect to differences in affecting the articular cartilage matrix molecules after intra-articular injections.

Betamethasone

Betamethasone is considered as the most short-acting corticosteroid in this paper. Usually betamethasone, compared from *TA* and *MPA* is injected intra-articular as a mixture of two type of esters. One water soluble called betamethasone sodium phosphate and one called betamethasone acetate which has a depot effect and a slow release (Bertone *et al.*, 2004). In a study by Knych *et al* (2017) it was shown that the concentration of betamethasone in synovial fluid was immeasurable in synovial fluid between 14 and 21 days after intra-articular injection.

In an *in vivo* study 12 horse received arthroscopically created osteochondral fragments on the distal radio carpal bone in both front legs. Two weeks after surgery all horses were intra-articular injected with betamethasone in one of the affected joints and the contralateral joint was injected with similar volume of saline. The treatment was repeated on day 35. The horses also were divided in to two groups. One group with six horses started an exercise program on a high-speed treadmill 17 days after surgery while the remaining horses were kept in box stables throughout the hole study. Day 56 post-surgery all horses underwent clinical examination and radiographically examinations before they were euthanatized. After euthanasia cartilage samples were collected for histological, histochemical and biochemical analysis. In the group of horses which had undergone the exercise program five of the six horses shown mild lameness. Four of the lame horses were lame in the control limb and the last one was lame in the treated limb. In the group with resting horses only five of the six horses could be evaluated since the last one got a septic arthritis and was therefore removed from the study. Of these five horses two were lame in the treated limb, two in the control limb and one in both the treated and the control limb. Neither in the exercised or in the resting group no significant histological difference was shown between cartilage from joints injected with betamethasone compared with joints injected with saline. In the group with exercised horses the histological changes were minimal worse in cartilage from treated joint compared with control joints but there was less reduction in safranin O staining in treated joints compared with control joints. The authors conclusions by this study was that no consistently severe detrimental effects associated with intra-articular injections with betamethasone in therapeutic dose (Foland *et al.*, 1994). Another study has on the other hand shown that betamethasone significantly decrease the synthesis of proteoglycans in articular cartilage unless only a low dose is used (Freaan *et al.*, 2002).

Triamcinolone acetone (TA)

TA also belongs to the group of short-acting corticosteroids, but it has a longer duration compared to betamethasone. It has been shown that *TA* is absorbed from synovial fluid rapidly after injection and 4 days after injection the concentration had significantly decreased. 15 days after injection the concentration of *TA* is undetectable in synovial fluid (Bertone *et al.*, 2004). *TA* seems to have a notable individual variation (Chen *et al.*, 1992).

Several *in vivo* studies have demonstrated the ability of *TA* to reduce lameness in horses with surgically induced damage in joint (Frisbie *et al.*, 1997; Kawcak *et al.*, 1998). According to the study by Frisbie *et al.*, 1997, *TA* except from reduce lameness also increased the concentration of HA and glycosaminoglycan in synovial fluid and decreased total protein and less inflammatory cell infiltration could be seen. These changes were not only detected in the treated joint, even in the control joint these positive effects could be seen (Frisbie *et al.*, 1997). Histomorphological parameters in articular cartilage also seems to be better after treatment with *TA* (Frisbie *et al.*, 1997; Kawcak *et al.*, 1998). In *in vivo studies*, it was shown based on histological examinations of cartilage samples treated with *TA* that *TA* can protect chondrocytes from the toxic effects caused by LPS (Bolt *et al.*, 2008). The same study also indicates that concentration of GAG content in cartilage was not affected after treatment with *TA*. The number of empty lacunae and pyknotic nuclei decreased after treatment with *TA* and stayed at decreased levels when the cartilage was exposed for LPS. If the number of empty lacunae first was

increased by treating the samples with LPS, *TA* could not reduce these negative effects (Bolt *et al.*, 2008).

In another *in vivo* study, it was shown that healthy joints, intra-articular injected with *TA* at recommended doses had an altered metabolism of articular cartilage already after one injection. Increased degradation of collagen type II and aggrecan could be seen but it was reversible and when it went back to normal again the degradation was even lower than it was before treatment (Céleste *et al.*, 2005).

Methylprednisolone acetate (MPA)

MPA is classified as a long-acting corticosteroid. In Sweden *MPA* are an uncommon alternative as treatment of joints with OA however it is frequently used in other countries, for example in the US and in the UK. After intra-articular injections *MPA* rapidly hydrolyze to the active moiety methylprednisolone (MP) (Bertone *et al.*, 2004). How long MP is detectable in synovial fluid after injection remains unclear. One study says 10 days (Lillich *et al.*, 1996), another on says 39 days (Autefage *et al.*, 1986) and a third on says 77 days (Knych., *et al* 2014).

To evaluate the effect of *MPA* at articular cartilage many studies have been done. A common trend in studies is that repeated high doses and repeated injections are used. One example is an *in vivo* study by Chunekamrai *et al.*, (1989) where eight horses without prior signs of lameness or OA were injected with 120 mg *MPA* per joint into the right radiocarpal and intercarpal joint once a week for eight weeks. Articular cartilage samples from treated joints indicated a decreased intensity of staining in safranin O fast green dye, which represent loss of GAGs in extracellular matrix. All samples from injected joints also showed chondrocyte necrosis, hypo cellularity and also loss of proteoglycans could be seen. The reduction of synthesis of proteoglycans last between 4 and 8 weeks after treatment.

When it comes to the effect on proteoglycan in articular cartilage after treatment with *MPA* it seems to depends on if the joint is healthy or not. An *in vitro* study where cartilage sample treated with interleukin-1 to mimic the conditions occurring during development of OA, IL-1 decreased the synthesis of proteoglycans but if the samples were treated with *MPA* the synthesis of proteoglycan increased (Yates *et al.*, 2006). On the other hand in cartilage samples from healthy joint only treated with *MPA* seems to have a decreased production of proteoglycan (Byron *et al.*, 2008). Also a study made for evaluate differences of acting of *MPA* in healthy joints compared to a damaged joints shows that *MPA* in a healthy joint decreased proteoglycan synthesis and the total protein was higher than in control joints. But if the sample first was treated with LPS to mimic a joint with OA the negative effects of *MPA* were not detected (Todhunter *et al.*, 1998).

DISCUSSION

In all the reviewed studies, there seems to be a general agreement that corticosteroids are effective in reducing lameness, which is the reason why it is so frequently used. Unfortunately, there is no study that compares *betamethasone*, *triamcinolone acetate* and *methylprednisolone acetate* to each other. Available studies have different study designs and are therefore not fully comparable.

Some studies are *in vivo* and some are *in vitro* which may give different results. For example, cartilage samples in *in vitro* studies lack the ability to eliminate the drug compared to a joint *in vivo*. A benefit with studies *in vitro* is on the other hand that it is much easier to get high reproducibility among samples which could be hard in *in vivo* studies since there are individual differences between horses. Another difference between studies is if the corticosteroid is injected in a healthy joint or a diseased joint with an inflammation already present. If the joint is injured there are also diverse ways to induce the damage. Since OA cause an imbalance between anabolic and catabolic mediators in the joint it is important to mimic this environment, which should be considered when selecting the method to induce the damage. For example, a surgically induced fragment may not cause this environment, which is important to remember when interpreting result from those studies.

Based on studies available today it is not possible to define which type of corticosteroid that is the best choice for intra-articular injection as treatment of OA, but some trends can be seen.

Betamethasone has the fewest number of studies and more studies would be desirable. Based on available studies *betamethasone* does not affect the cartilage in any adverse way. One study showed reduced synthesis of proteoglycans and GAGs in cartilage after treatment with *betamethasone* (Frean *et al.*, 1994). On the other hand, another study by Foland *et al.*, (1994) shows that this negative effect only could be seen in articular cartilage from horses which underwent daily training as soon as three days after treatment. This indicate that *betamethasone* does not alter the articular cartilage in an adverse way if the horse is taken out of training while the drug is acting. Since *betamethasone* is a short-acting corticosteroid, the time out of training after treatment does not need to be so long and therefore, it is a good alternative for athletic horses.

Triamcinolone acetonide is the only corticosteroid in this essay, which has shown positive effect on the cartilage. In the study by Frisbie *et al.*, (1997), it was seen that *TA* can protect chondrocytes from stress and the total protein and the number of inflammatory cells were also decreased after treatment with *TA* (Bolt *et al.*, 2008). On the other hand, some studies insist on increased degradation of collagen type II and aggrecan (Céleste *et al.*, 2005). The alteration which can be seen after treatment is reversible. When the changes induced by *TA* returns to normal, the degradation of articular cartilage can be lower than before treatment and therefore, *TA* is a good alternative as treatment of OA. Since *TA* is capable to protect chondrocytes from stress, it is a particularly good choice in an early stage of OA.

Another interesting fact with *TA* is that the positive changes which have been seen after treatment with *TA*, such as decreased total protein and decreased number of inflammatory cells not only could be seen in the treated joint. The same changes were also seen in the control joint, which indicate a systemic spread of the drug (Frisbie *et al.*, 1997).

The type of corticosteroid which has shown most devastating effects on articular cartilage is *methylprednisolone acetate*. Both reduced staining with safranin O fast green dye which indicates loss of GAGs, chondrocyte necrosis, hypo cellularity and loss of proteoglycans have been seen after treatment with *MPA*. These changes were observed as long as 4-8 weeks after last injection (Chunekamrai *et al.*, 1989). In the study by a Chunekamrai *et al.*, (1989) high

doses and frequent injection . Recommended dosage in Sweden is 40-120 mg per joint and it should be enough with one injection (Läkemedelsverket, 2010). In the study by Chunekamrai *et al.*, (1989) the maximum dose, 120 mg per joint, is injected weekly for eight weeks. It should be interesting to see if the same detrimental effects on the articular cartilage would occur even if the horses were injected with a lower dose and only once instead of once a week for eight weeks.

Something interesting with *MPA* is that it seems like it alters the synthesis of proteoglycans different in healthy and non-healthy joints. In healthy joints decreased syntheses of proteoglycans have been observed after treatment (Byron *et al.*, 2008). But both if the cartilage is stimulated with IL-1 or LPS, treatment can help to increase the reduced synthesis caused by IL-1 (Yates *et al.*, 2006) or LPS (Todhunter *et al.*, 1998). This information makes the study by Chunekamrai *et al.*, (1989) even more irrelevant since the horses in that study were healthy and had no prior signs of lameness or OA.

MPA should not be the first choice as treatment of OA since it is a long-acting corticosteroid and therefore alter the metabolism of the extracellular matrix for a long time after injection. Treatment with *MPA* requires considerably longer time out of training compared to *betamethasone* or *TA* and therefore it is not the best alternative for athletic horses since this horse requires treatment which makes them able to be back in training as soon as possible. If a horse does not respond to other treatments, *MPA* could be an alternative if the owner understands that the horse needs a long time of rehabilitation after treatment. *MPA* could also be an alternative for hacking horses, which are not exposed for exercise in the same incidence as athletic horses.

Based on this literature review corticosteroids can be a good alternative to treat OA if they are used correctly. So, what is meant by correct use of corticosteroids? First it is important to find the joint in which OA is developing in an early stage. *TA* can protect chondrocytes from stress so if a joint get an injection soon after the disease starts it can restore the balance between catabolic and anabolic processes. Good diagnostic is also an important thing. It is of great importance that the veterinarian only injects inflamed joints since it has been shown that corticosteroids affect the articular cartilage in different ways depending on the health status of the joint. It is of great concern that the horse is subject to strict rest after treatment and that the veterinarian has in mind that the horse may show up clinically healthy a few days after the injection because of the strong anti-inflammatory effects of corticosteroids. The cartilage still needs time to advert to normal function again and therefore, rest and time is essential for good long-term results of treatment.

One difficult thing with corticosteroids is that you cannot accurately know how long they affect the joint. It is possible to see when it is undetectable in synovial fluid but that does not automatically means that the effect is gone since corticosteroids affect the genome and because of that can exert a longer effect. More pharmacokinetic and pharmacodynamics studies about corticosteroids are required. The fact that it is unknown how long corticosteroids affect the joint makes it difficult to know for how long the horse need to be out of training after intra-articular treatment with corticosteroids.

Even if there are a lot of questions left about corticosteroids, it is important to remember that we today do not have better options for treatment of OA. The discussion about corticosteroids negative effects on the articular cartilage makes many horse owners scared and reluctant to treat their horses intra-articular with corticosteroids. It cannot be excluded that corticosteroids do not affect the articular cartilage in an adverse way but it is absolutely certain that the cartilage will get damage if the inflammation in joint is not inhibited.

In conclusion, there are no strong indications that articular cartilage becomes damaged after treatment with corticosteroids as long as the dosage is correct and the horse rest while the drug is acting. Further studies are required to get a better understanding of the use of corticosteroids, and comparative studies between different types of corticosteroids are desirable. Meanwhile, there is no reason to change the general view that corticosteroids are a good alternative for treatment of OA in horses.

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