CURRENT ASPECTS OF HYALURONATE TREATMENT IN KNEE OSTEOARTHRITIS

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Abstract: *Osteoarthritis (OA) involves pathological changes in all joint tissues, including cartilage degradation and synovitis. Synovial inflammation is significantly associated with pain severity and incidence of OA. Apparently, synovitis also plays an active role in the initiation and progression of cartilage erosion in gonarthrosis by direct secretion of catabolic enzymes as well as factors that stimulate the catabolic activity of chondrocytes. New studies demonstrate the presence of cytokines and chemokines in the articular synovial fluid. Among arthroses, the incidence of knee localized OA is the largest. In early stages, first-line treatment consists of adjusting the lifestyle, weight loss recommendations, symptomatic treatment with non-steroidal anti-inflammatory drugs and intra-articular (IA) administration of corticosteroids (CS). Intra-articular injection therapy with hyaluronic acid (IA HA) is a viable option after using the first-line methods. Efficacy of hyaluronan is debated even 20 years after its implementation on the world market. Recently conducted systematic reviews and meta-analytical studies compare the effectiveness of IA HA injections with placebo treatment. Results advocate for hyaluronic acid.*

Etiology and diagnosis of knee osteoarthritis

Osteoarthritis (OA) is classified as primary (idiopathic) or secondary. In both cases, the hyaline articular cartilage is mainly affected, causing knee arthritis. Frequently encountered etiologies in secondary OA are (a) post-traumatic, (b) congenital or due to anatomical malformations of the tibio-femoral joint, (c) postoperative, (d) metabolic, (e) due to endocrine disfunctions and (g) aseptic osteonecrosis.(1) Even with the general consideration that knee OA is a disease of the elderly, the diagnosis is established on average around the age of 55 years.(2) According to Prieto et al. the maximum incidence of OA is around 70-75 years, and the risk of developing knee OA is double in women over 55 years, compared to those under 40.(2,3)

OA is characterized by the following main pathological events:

- progressive degeneration of cartilage
- subchondral bone remodelling
- osteophytes development
- synovial inflammation

OA is classically considered a consequence of the "wear and tear" effect, originally described in 1882 by August Weismann as the result of excessive use of the body at cellular level, without the ability to ultimately compensate the produced damage. Subsequently, the etiology and pathogenesis of OA was attributed to excessive mechanical stress force applied in the context of a systemic susceptibility. Knee loading due to obesity produces mechanical stress on the articular surface and leads to bone and cartilage destruction.(4) Cartilage destruction by foreign body reaction of synovial cells causes inflammation of the synovial membrane with the release of inflammatory mediators in the joint space. This mechanism is considered the original and primary trigger mechanism of OA.(5,6,7) Research on synovial fluid has revealed the existence of inflammatory cytokines that may be involved in stimulating destruction of cartilage and other soft tissues of the knee joint, e.g. menisci, ligaments and periarticular muscles. It is considered that these cytokines and chemokines are secreted by aged chondrocytes.(8) Past injuries (rupture of anterior cruciate ligaments, meniscal injuries or fractures, etc.) are fertile ground for the emergence of OA.(8) In a prospective study of over 1.300 adults with 36 years of clinical follow-up, the relative risk of developing osteoarthritis was five times higher in patients who suffered a knee injury during follow-up compared to those without injuries.(9)

Major diagnostic tools are medical history, general physical examination, local examination, imaging investigations, and laboratory tests. In a patient with history of OA a dull joint pain is often found after the patient begins to walk.

Specific characteristics of a patient with OA:(10)

- *Pain*
  - pain when beginning to walk
  - pain during walking
  - a permanent pain at night
  - a need for painkillers
- *Loss of function*
  - rigidity
  - limited range of motion
  - limitation on daily activities
  - need for orthopedic crutches
- *Other symptoms*
  - crepitations
  - increased sensitivity to cold

Physical examination follows the classical hierarchy: local

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examination consisting of inspection and palpation of the region, range of motion in flexion and extension and specific functional tests (ex. anterior and posterior drawer test, stress valgus/varus test).

The Kellgren radiological classification of OA from 1957 is still used:(11,12)

- Grade I: questionable joint space narrowing and possible osteophytes
- Grade II: questionable joint space narrowing with well-defined osteophytes
- Grade III: joint space narrowing with multiple osteophytes, well-defined areas of sclerosis and possible deformity of bone ends
- Grade IV: extreme joint space narrowing, large osteophytes, sclerosis and severe bone deformity of bone ends

The currently most popular classification of OA was described by Swedish radiologist, Sven Ahlbäck in 1969:(13)

- Grade I: joint space narrowing (less than 3 mm)
- Grade II: joint space obliteration
- Grade III: minor bone attrition (0-5 mm)
- Grade IV: moderate bone attrition (5-10 mm)
- Grade V: severe bone attrition (more than 10 mm)

General treatment of osteoarthritis

Most patients diagnosed with OA for the first time will initially undertake a non-pharmacological treatment.(14) Conservative treatment includes recommendations on weight management, physical therapy or physiotherapy, hydrotherapy and walking with assistive devices.(14) Acetaminophen and oral or topical NSAIDs are recommended as first line pharmacological treatment.(15) According to guidelines from 2014 Osteoarthritis Research Society International (OARSI) all patients diagnosed with OA have the following recommendations:(16)

- land-based exercise
- weight management
- strength training
- water based exercise
- self management and education

Non-surgical treatment of OA is classified by OARSI based on the existence of associated comorbidities and concomitant disease on multiple joints. Thus, in patients with OA in one knee and without comorbidities the following are recommended:(16)

- Biomechanical interventions
- Intra-articular corticosteroids
- Topical NSAIDs
- Walking cane
- Oral COX-2 inhibitors (selective NSAIDs)
- Capsaicin
- Oral non-selective NSAIDs
- Duloxetine
- Acetaminophen (Paracetamol)

For the category of patients who associate relevant comorbidities (renal failure, hepatic failure, history of stroke, hypertension) the following are recommended:(15)

- Topical NSAIDs
- Intra-articular corticosteroids
- Walking cane
- Capsaicin
- Biomechanical interventions

In addition to conservative treatment in patients with multiple joint involvement and associated comorbidities, OARSI recommended balneotherapy. OARSI also recommends considering open orthopedic surgery if more conservative treatment modalities are ineffective.(16)

Hyaluronan – short description

First discovered in 1934 by Karl Meyer in the vitreous humor of the eye and subsequently synthesized in vitro in 1964 (17), hyaluronan (HA) was originally used in eye and aesthetic surgery. Later, between 1970 and 1980 it became the subject of more and more human and veterinary clinical studies, being used to treat OA in racing horses. Alexander Endre Balazs was known for doing extensive research about hyaluronan an putting it up for sale in 1980 in Sweden, under the name of "Healon", produced by Pharmacia AB. Initially used in ophthalmology, HA was implemented in the form of intra-articular injection in the treatment of OA of the knee in 1986 under the name of Hyalart / Hyalgan manufactured by Fidia in Italy. In 1986 the name of hyaluronic acid was changed to "hyaluronan" because of its polysaccharide structure and its synthesis by mammalian cells as a salt, not as an acid. Nowadays, hyaluronan treatment is globally used as adjuvant therapy in knee OA and other disorders. Hyaluronate is a nonsulfated, negatively charged glycosaminoglycan (GAG) with a molecular weight that varies between 6500 and 10.900 kDa in the synovial fluid.(18) It has a simple structure compared to other GAGs and is composed of alternating units of N-acetylglucosamine and glucuronic acid. It is described as existing in all vertebrate tissues and body fluids but also in those of bacteria.(19) The presence of HA in synovial fluids explains the available thixotropic characteristics. Secretion of endogenous HA occurs at the level of the synovial membrane in joints. Histologically, the synovial membrane is composed of cell types A, B and C. A type cells are known as macrophages, B-type cells are fibroblasts.(20) It is believed that the C cells carry out an intermediate function.(20) B cells are secreting the endogenous hyaluronate found in the synovial fluid. Revell et al. claims that the number of A and B cells increases in OA, forming a vicious pathologic circle.(21) Therefore, B cells secrete HA with protective purposes and macrophages stimulate cartilage destruction.(21) HAs core effects are lubrication of the inter-articular spaces and shock absorption (22).

Mechanism of action

In the US, there are seven FDA approved HA products for intra-articular (IA) injection use and the primary ingredient of these injections is sodium hyaluronate, containing quantities of about 20-25mg/injection with a molecular weight that varies between 500-6500 kDa.(23) HA can be obtained by biological fermentation or derived from avian products, the first having superior effects according to recent trials.(24) The main mechanism of action is based on its lubricating and shock absorbing properties. The conclusion of a meta-analysis performed on 104 trials is that the three main events involved in the mechanism of action of IA HA are chondroprotection, inhibition of inflammatory mediators and stimulation of proteoglycan and glycosaminoglycan synthesis.(24) Chondroprotection is achieved by HA binding to cluster of differentiation 44 receptors (CD44) thus leading to a lowering effect of chondrocyte apoptosis and stimulation of chondrocyte proliferation.(25) By binding to CD44 HA inhibits the expression of IL-1β resulting in a decline in matrix-metalloproteinase 1, 2, 3, 9 and 13 (MMP-1, MMP-2, MMP-3, MMP-9, MMP-13) formation.(26,27) Chang has shown that binding of HA to CD44 receptor on viscosupplementation with a HA of high-molecular-weight has better effects compared to a product with low-molecular-weight, thus sustaining the new high-molecular weight products on the market (e.g. GO ON - Rottapharm).(28) On the other hand, it was demonstrated that HA products with a 500-730 kDa (e.g. Hymovis – Fidia) pass

through the synovial membrane.(29,30) Therefore, endogenous synthesis of HA is stimulated leading to a large quantity of high molecular HA to be secreted in the synovial liquid.(29,30) A negative regulator of IL-1β called mitogen-activated protein kinase phosphatase (MKP) is responsible for the inhibition of IL-1β in the synovial fluid after binding of HA to CD44 receptor.(31) Chondrocyte apoptosis is suppressed by certain proteases called disintegrines and metalloproteinases with thrombospondin motifs (ADAMTS proteinases), which are also involved in the molecular destruction of important components of the synovial fluid such as aggrecan, brevican and versican.(32) Reactive oxygen species such as nitric oxide (NO) are also involved in the destruction of cartilage by stimulating chondrocyte apoptosis in synovial fluid.(33) In two different studies, Peng and Takahashi showed that existing HA reduces oxidative stress in the joint tissues by suppressing the production of NO and inhibiting the expression of IL-1β.(34,35) In a comparative study on human synovial cells, Yasui demonstrated that high molecular weight products have a higher rate of prostaglandin E2 (PGE2) inhibition. The chondroprotective effect of PGE2 inhibition is due to the same interaction with CD44 receptor.(36) Experimental studies on animal models have demonstrated that IA-HA has stimulating effects on the synthesis of proteoglycans and GAGs, thus delaying the progression of OA.(37,38) The main proteoglycan found in the synovial fluid is aggrecan and two studies have concluded that intra-articularly HA treatment has a suppressive effect on the degradation of aggrecans and a stimulating effect on the intrinsic development of proteoglycans, thus slowing down the development of the disease.(39,40) A marker of proteoglycan synthesis, sulfate (35SO4), is considered to be incorporated into chondrocytes after injections with HA.(41) This therapy does not only supplement the joint space with exogenous HA, but also promotes the production of intrinsic HA.(42) Inhibitory effects of articular pain receptors, direct anti-inflammatory effects and activity shrinking of matrix metalloproteinases (MMPs) were also described.(43) IA-HA administration proved to inhibit the expression of MMP-3 (stromelysin) resulting in reduced synthesis of MMPs and thus, a decrease in joint cartilage destruction.(23) Inhibition of MMP-3 provided by HA induces a blocking of cartilage proteoglycans and collagen type II degradation.(23) In grades II and III of OA, treatment with HA resulted in inhibition of IL-1β which is also responsible for expression of MMP-3 and formation of prostaglandins.(44,45) Also, IL-1β inhibits and reduces the formation of extracellular cartilage matrix.(46) IL-1β is a key mediator in the inflammatory effects of HA and is regulated by binding to CD44 receptors afore mentioned.(47,48) High-molecular-weight HA has the ability to suppress numerous inflammatory mediators by binding to Toll-Like receptor 2 and 4 (TLR 2 and 4), including TNF-α, IL-1 β, IL-8, IL-17, MMP-13.(49) The mechanical effects of HA are important in its mechanism of action. It is considered that knees affected by OA maintain a higher friction between cartilages compared to a normal knee and HA treatment counteracts this by its lubricating and shock absorption properties. Due to their viscosity, products with a high-molecular-weight showed superior results in terms of inter-articular friction.(50) A study of analgesic effects of IA-HA treatment demonstrated that HA does not bind to bradykinin receptors such as classical analgesics, but exerts a direct effect on free nerve endings and HA receptors within the joint tissue.(51) Peña Ede et al. demonstrated in vitro that the analgesic effect of HA occurs at the level of stretch-activated-ion-channels, through mechanosensory mechanisms having their activity significantly reduced after HA binds to its receptors.(52) His team also confirmed that low-molecular-weight HA has reduced effectiveness. Briefly, the mechanisms of action of endogenous and exogenous HA ultimately aim to renovate metabolic homeostasis of intra-articular synovial fluid flow and improve pain relief.(23)

Hyaluronate in osteoarthritis

Even after 20 years of use, the effectiveness of treatment with HA is considered by many authors as uncertain and insecure. Viscosupplementation with IA HA is administered in order to protect cartilage matrix components, cell cartilage functionality and synovial tissue from mechanical stress and impose its analgesic effects on nociceptors in the affected joints.(53) Multiple meta-analytic studies and reviews in the current literature are trying to bring sustaining evidence. On a group of 253 patients diagnosed with Kellgreen grade II and III OA, Chevalier concluded that a single injection of 6 ml of IA HA (Hylan GF-20) brought significant clinical improvements compared to placebo.(54) In a meta-analytic study conducted between 1966 and 2012, which included 89 clinical trials, Rutjes et al. showed that studies where a high-molecular-weight HA was injected had superior results in comparison to those where of low-molecular-weight HA was used (55), thus opening the debates and discussions regarding the molecular weight of HA. Another study compared the effects based on the molecular weight of HA used and concluded that symptoms were significantly improved after the injection of high-molecular-weight HA (GO-ON; kD MW 800-1500, 25 mg/2.5 ml) compared to the low-molecular-weight HA (Hyalgan; GM500-730 kD, 20 mg/2 ml).(56) Changes in walking speed, cadence, stride length, gait cycle percent and single support leg were all higher 6 months after injection of IA-HA.(57)

Hyaluronate and corticosteroids

Corticosteroids (CS) have anti-inflammatory and immunosuppressive effects with a complex mechanism of action, acting on nuclear steroid receptors. They inhibit phagocytosis, production of neutrophils, metalloproteinases and prevent synthesis of inflammatory mediators such as prostaglandins and leukotrienes and at the same time, they decrease vascular permeability and inhibit the accumulation of inflammatory cells.(58)

IA used CS are:

- Methylprednisolone acetate
- Betamethasone acetate/ sodium phosphate
- Triamcinolone acetate/ hexacetonide
- Dexamethasone

Skwara compared the effects of IA triamcinolone with IA HA analyzing gait, maximum vertical force, muscle activity and pain. The results showed that both have similar effects and IA-HA was not superior to triamcinolone.(59) In an analysis of seven trials, comprising 606 patients, the effectiveness of IA administered CS was superior to that of IA HA at 4 weeks post-injection.(60) In contrast, at 8 weeks, the group treated with HA had significantly better results.(61) In 2014, Leighton et al. compared the two treatments on 441 patients and concluded that effects on pain relief are similar in both treatment options with the results of the group treated with hyaluronic acid not inferior to those treated with CS.(62) In a randomized, double-blind study conducted over a period of 6 months in 25 centers in France, UK, Canada, USA, and Germany, Housman found that both regimens were effective in relieving pain, but without the existence of a significant difference between the two methods, thus contesting the hypothesis of HA superiority.(63) In another study conducted in Japan on a total of 51 patients, Shimizu followed levels of inflammatory biomarkers after injection of IA HA and CS. The group treated with IA HA showed lower levels of MMP-9 ($p = <0.01$), supporting the MMPs-inhibitory theory of HA. Other inflammatory markers were analyzed

(chondroitin-6-sulphate, chondroitin-4-sulphate, tissue factor pathway inhibitor and MMP-1) but with no significant differences in outcomes between the two groups.(64)

Hyaluronate and platelet-rich-plasma

In competition with IA HA as effects and usage in treating OA, are platelet-rich-plasma (PRP) injections. PRP is obtained by autologous centrifugation of blood (65). Thus, a concentrated fluid is obtained with approximately 5 times more platelets than normal plasma.(65,66) The platelet-rich concentrate is activated by adding calcium chloride leading to the release of growth factors (insulin-like growth factor, transforming growth factor β-1, platelet derived growth factor) and bioactive molecules (cytokines, chemokines, arachidonic acid metabolites, extracellular matrix proteins, nucleotides, ascorbic acid).(66,67) Kon et al. compared PRP with IA HA on a group of 150 patients diagnosed with OA. Treatment with IA HA had superior results with regard to pain relief, pain, symptoms remission and recovery of joint function 6 months after injection.(68) Results were favoring PRP in young active patients with light degenerative disease and IA HA in elder patients with severe cartilage degeneration (68). Filardo concluded in a randomized study conducted on 192 patients that PRP does not provide clinical improvement superior to HA and therefore should not be preferred over HA injections.(69) Sundman et al. compared the effects of HA and PRP on inflammation by measuring levels of TNF-α, IL-6 and IL-8. Both treatments decreased the production of TNF- α; IL-6 levels decreased only in the HA group;(70) these results suggest that both treatments influence inflammation by different mechanisms. In a review published in 2014, Andia proposes a new approach in the treatment of OA which involves combining the two substances in a single product, concluding that additional randomized trials using HA and PRP in comparison are required in order to provide evidence on the impact of this approach.(71)

International guidelines in treatment with hyaluronic acid

Treatment with HA has been considered by OARSI 2010 guidelines as having reserved effects on pain relief in patients with knee or hip OA.(15) Recent updates indicate that treatment with HA does not show superior results compared to placebo treatment, thus IA injections are not recommended by OARSI as initial therapy.(16) Similar to OARSI, The American College of Rheumatology claims in 2012 that IA HA is indicated when multiple conservative treatment methods did not provide positive results.(14) Due to lack of concrete scientific evidence OARSI 2014 guidelines deem IA HA as having uncertain effects in OA of the knee and other joints.(16)

CONCLUSIONS

Recent literature indicates that intra-articular HA injections positively affect the quality of life of patients and the level of satisfaction after treatment. Even if usage of IA HA is not indicated as first-line treatment by OARSI guidelines, results achieved on mild to severe OA by injecting high-molecular-weight IA HA proved to be subjectively positive for patients. Being a new concept, the number of studies conducted on this subject is still small; therefore, recommendations can not be developed. From our experience, treatment with IA HA is preferred by patients that have to choose between conservative and surgical treatment. CS showed stronger positive effects over a short period of time, becoming inferior to HA with regard to pain relief and physical joint functionality for extended periods of time. PRP treatment is superior to HA only in young patients with OA and in early stages, even over a long period of time. Multiple studies comparing various new products and concepts are needed to clarify the indications, recommendations and guidelines for intra-articular treatment of osteoarthritis with hyaluronic acid.

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