

Intra-articular 500-730 kDa hyaluronan (Hyalgan®) therapy in the management of osteoarthritis. Can a specific therapeutic profile be defined?

D. GUIDOLIN

Department of Neuroscience, University of Padua, Padua, Italy

Abstract. – **OBJECTIVE:** Intra-articular hyaluronic acid (HA) injections for the symptomatic relief of pain have been available for treatment since the 1980s. Practitioner experience and real-world evidence have been accumulated to suggest that HA injections are effective and well tolerated in patients. Treatment guidelines issued by different professional medical societies, however, do not point in a single direction. This appears mainly due to conflicting results of the proposed meta-analyses at least in part associated with a variability between different HA preparations on different outcome parameters, suggesting that intra-articular HA products should not be treated as a group, as there are differences between them influencing both efficacy and safety.

PATIENTS AND METHODS: The present review is focused on the quite relevant amount of preclinical and clinical studies (the first studies dating back to thirty years ago) concerning a specific HA-based preparation (500-730 kDa native HA) and supporting its use as a tool for intra-articular therapy. They also include comparative studies to other HA preparations.

RESULTS: The analysis of this experience allows to define a specific profile for 500-730 kDa HA as a tool for the management of osteoarthritis in terms of main mechanism of action, kinetics features and interaction with joint tissues, subpopulation of patients expected to obtain the highest benefit from the treatment, safety issues and impact on disease-cost.

CONCLUSIONS: The abovementioned factors may also represent useful criteria to better characterize the specificities of each HA-based preparation and to achieve a more stratified categorization of this class of therapeutic tools.

Key Words

Osteoarthritis, Hyaluronic acid, Pharmacology, Mechanism of action.

Introduction

The idea that injections of hyaluronic acid (HA) into the joint cavity could reduce pain and improve mobility emerged at the beginning of the 1970s when studies were published showing that the use of HA as a therapeutic agent in osteoarthritis (OA) of the human knee^{1,2} was leading to statistically significant improvement in many functional variables of the articular joint. This proposal was based on the peculiar physicochemical properties of HA arising from its unique macromolecular structure, an exceptionally long chain (up to 30 μm) of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. Despite the simplicity of its primary structure, this linear polysaccharide can adopt highly coiled conformations in solution leading to the formation of extensive macromolecular entanglements and networks that confer to HA solutions their characteristic rheological properties in terms of elasticity and viscosity. In the organism, the largest single reservoir of HA is the synovial fluid (SF) of the diarthrodial joints, where the HA molecules are mainly synthesized by the type B synoviocytes, releasing a polydispersed HA population with molecular weight (MW) in the range between $2 \cdot 10^6$ and $10 \cdot 10^6$ Da and concentrations of 0.5-4 mg/mL ³⁻⁵. The high concentration of HA in SF is essential for normal joint function, because HA confers to SF exceptional viscoelasticity and lubricating properties, responsible for shock absorption under conditions of high compression or shear, and lubrication in low load states. These unique non-newtonian rheological properties of HA not only reduce wear and attrition of articular cartilage during joint motion⁶⁻⁸ but also stabilize joints at low shear rates⁹. It is well known that joint arthrop-

athies of traumatic and degenerative nature (such as osteoarthritis) are associated with a reduction of the molecular weight and concentration of hyaluronan in the synovial fluid. In fact, the presence of proinflammatory cytokines, free radicals and proteinases in the synovia can adversely affect the metabolism of the lining type B fibroblasts, leading to the biosynthesis of HA with abnormal MW, as has been shown by analysis of synovial fluid from pathologic joints^{5,7,10}. In addition, HA also may be depolymerized by oxygen-derived free radicals¹¹ and intracellularly by hyaluronidases, and other glycosidases from synoviocytes and leukocytes in the synovium^{12,13}. The decline in HA molecular size coupled with its dilution by infiltration of plasma fluid and proteins (caused by increased synovial membrane permeability) reduce the rheological properties of synovial fluid from diseased joints^{5,7,10,11}. As a consequence, it was contended that cartilage attrition and subchondral bone remodeling was enhanced contributing to progression of pathology and clinical symptoms.

Thus, more than thirty years ago Balazs and Denlinger¹⁴ introduced the concept of *viscosupplementation*, a therapeutical approach to OA involving the replacement of the SF with highly purified HA to restore (or supplement) SF viscoelasticity, to decrease symptoms, and improve joint functionality.

In the years that followed, intra-articular injection of HA-based preparations gained consensus among practitioners who recognized this approach as a safe and effective treatment of OA, safety being a major issue¹⁵⁻²¹. A Cochrane meta-analysis¹⁷ of 76 trials showed that this approach is effective in OA of the knee with beneficial effects on pain, function and patient global assessment, a finding confirmed by more recent meta-analyses²².

Therefore, as briefly summarized in Table I, this treatment modality was in general accepted in the guidelines²³⁻³⁸ concerning the management of OA, but not without debate as evidenced by the lack of recommendation in the NCC-CC (2008) and in the AAOS (2013) clinical guidelines or the 'uncertain' rating obtained in the OARSI (2014) statement. This appears mainly due to conflicting results of the proposed meta-analyses³⁶ that may arise from methodological differences or from flaws (the AAOS 2013 document, for instance, was quite criticized from a methodological point of view: see³⁹ for a thoughtful discussion of the topic).

In this respect, however, a particular point likely deserves consideration. As emphasized in Table I, almost all the published analyses did not differentiate among HA-based products. All of them were usually considered as a class of compounds (called 'i.a. HA', 'viscosupplements' and similar) sharing common properties. Instead, the currently available HA-based preparations for

Table I. Intraarticular HA therapy in the guidelines on the management of OA.

Guideline	Pathology	HA preparation	Recommendation	Reference
ACR 2000	Knee, hip OA	i.a. HA	Yes	[23]
EULAR 2003	Knee OA	i.a. HA	Yes	[24]
EULAR 2005	Hip OA	i.a. HA	Yes	[25]
EULAR 2007	Hand OA	i.a. HA	Yes	[26]
NCC-CC 2008	Knee, hip OA	i.a. HA	No	[27]
OARSI 2008	Knee, hip OA	i.a. HA	Yes	[28]
OARSI 2009	Knee, hip OA	i.a. HA	Yes	[29]
MQIC 2011	Knee, hip OA	i.a. HA	Yes	[30]
ACR 2012	Hand, hip, knee OA	i.a. HA	Yes	[31]
AAOS 2013	Knee OA	i.a. HA	No	[32]
ESCEO 2014	Knee OA	i.a. HA	Yes	[33]
OARSI 2014	Knee OA	i.a. HA	Uncertain	[34]
ACR 2014	Knee OA	i.a. HA	Yes	[35]
European consensus task-force 2015	Knee, hip, shoulder, ankle, hand	i.a. HA	Yes	[36]
US systematic review 2015	Knee OA	i.a. HA	Yes	[37]
AMSSM 2016	Knee OA	i.a. HA	Yes	[38]

AAOS: American Association of Orthopedic Surgeons; ACR: American College of Rheumatology; AMSSM: American Medical Society for Sport Medicine; EULAR: European League Against Rheumatism; ESCEO: European Society for Clinical and Economic aspects of Osteoporosis and osteoarthritis; NCC-CC: National Collaboration Centre for Chronic Conditions; MQIC: Michigan Quality Improvement Consortium; OARSI: Osteoarthritis Research Society International.

intra-articular administration significantly vary in concentration, molecular weight and protocol of injection. Furthermore, they can also differ in terms of molecular organization including solutions of native HA, as well as materials chemically derived from HA (such as hylans⁴⁰ and hyadd⁴¹), engineered to increase elastoviscosity and intra-articular residence time. Thus, possible differences in the efficacy profile specific to each product, may affect the results of the statistical analyses performed on the category as a whole⁴². This may help explain different views on the HA-based therapies in OA. A further consequence of this type of approach was pointed out by Migliore and coworkers in a two-parts study aimed at identifying scientific evidence from preclinical⁴³ and clinical⁴⁴ studies supporting the use of intra-articular HA marketed in Italy. It was observed that only a quite limited number of branded formulations were supported by reports providing scientific evidence, while the majority remained without direct proof. In other words, the rationale of use of these products was based on their nature, as if a class effect existed such that all HA-based preparations would yield similar effects and study results from a certain preparation could be extended to other HA-containing products that differ in composition. Thus, it has been suggested that larger and brand-specific studies should be provided to guide clinicians in making an appropriate choice regarding HA-based intra-articular therapy.

A specific HA preparation (based on 500-730 kDa native HA, and branded as Hyalgan[®], Hyalart[®], Hyalactin[®]) is the focus of the present review, since it is characterized by a relevant amount of preclinical and clinical studies supporting its use as a tool for intra-articular therapy, the first studies dating back to thirty years ago^{45,46}. Furthermore, it has also often used as a reference product in studies aimed at defining the efficacy profile of other HA-based preparations⁴⁷⁻⁴⁹. Thus, the analysis of this experience may be of help in the identification of the parameters that could be evaluated to characterize specific properties of each given HA-based preparation in order to allow a better positioning of each product in the framework of the available therapeutical strategies.

Early Evidence

The first clinical study performing intra-articular administration of 500-730 kDa native HA (Hyalgan[®]) in OA of the human knee was reported by Dixon and coworkers in 1988⁴⁶. The study was

a placebo-controlled study involving three hospital centers in the UK, and sixty-three patients (30 HA, 33 placebo) entered the trial. After the first 2 ml injection of 20 mg HA or placebo, they were seen again for reassessment and further injections at intervals of 1, 2, 3, 5, 7, 9, 11, 15, 19 and 23 weeks. A final assessment was performed at week 48. At each visit, gradings of the severity of pain at rest and pain on movement were assessed using visual analogue scales (VAS), while an 8-point scale was used to evaluate the 'Activities of daily living' (ADL). The results showed that the applied treatment was well tolerated and indicated significant reductions in joint pain, both at rest and on movement, thereby giving support to the hypothesis that this type of treatment was clinically beneficial. In the years that followed more placebo-controlled trials on knee OA^{50,51} became available. In these trials, patients received 4⁵⁰ or 5⁵¹ intra-articular injections of 20mg/2ml 500-730 kDa HA (or placebo) at weekly intervals. The results confirmed the previously reported data, showing that the treatment was able to significantly improve pain and functional status in patients with knee OA. These studies, however, provided additional relevant information. The onset of the beneficial effect was found to be gradual, becoming evident by the third week, but long-lasting. In the study by Dougados et al⁵⁰, for instance, the therapeutic benefit of the treatment was still present at one year. During the 1-year follow-up the need to perform additional local therapy (joint fluid aspiration during hyarthral episodes, local corticosteroid injection) was significantly less frequent in the HA-treated group than in the placebo group, and after one year the clinical judgement and the improvement in the functional index were significantly more favorable in the treated group than in the placebo group.

In the same period the biophysical features of this HA solution were also carefully studied, since, according to the *viscosupplementation* concept, a substance able to restore the viscoelastic properties of the SF should exhibit a similar mechanical behavior. SF acts predominantly as a viscous fluid when it is exposed to low deformation frequencies (slow movement) and behaves as an elastic shock absorber when it is subjected to a high rate of deformation, such as during running or jumping⁵². This rheological profile, which is strongly dependent on the HA content, is critical to the physiologic function of the synovial fluid. It can be characterized by evaluating how the frequency of a properly applied mechanical stress affects the relative values of

the elastic modulus and the viscous modulus^{53,54}. The strain frequency at which these two moduli intersect is called the “cross-over point” (Figure 1A) and represents the frequency (~ 0.16 Hz in healthy SF) at which the SF changes from predominantly viscous to predominantly elastic. These rheological properties depend on both the molecular weight and the concentration of HA and the cross-over frequency typically moves to higher values as solutions become more dilute and structure disappears. Thus, when compared to healthy SF, the cross-over frequency exhibited by 500-730 kDa HA at the concentration used for intra-articular therapy resulted significantly higher^{55,56} indicating a quite different rheological behavior. Moreover, it must be pointed out that endogenous HA is also involved in the lubrication of the synovial joint by two main

mechanisms. From one side it is characterized by intrinsic lubricating properties⁵⁷, on the other side it can interact with phospholipids, giving rise to complexes exhibiting peculiar lubricating⁵⁸ and protective⁵⁹ characteristics. Both these features, however, are MW-dependent and become more efficient with increasing chain length^{58,59}. Thus, the biophysical properties of 500-730 kDa HA cannot support an explanation of the above mentioned clinical data simply in terms of ‘viscosupplementation’. In addition, data on the kinetics of HA with MW in this range indicated that the half-life and residence time following a single intra-articular injection were of about 16 and 60 hours respectively⁶⁰, a finding inconsistent with the long-lasting effect observed. A key question, therefore, was raised concerning the mechanism of action of this HA-based material.

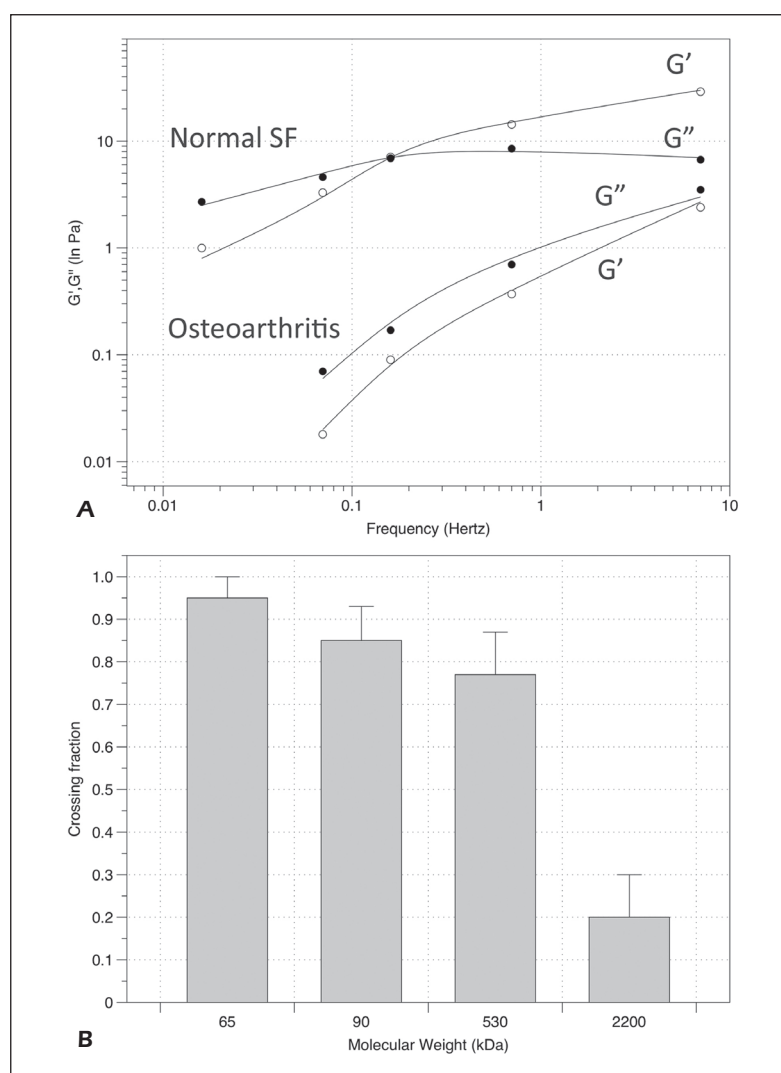


Figure 1. Viscoelasticity of hyaluronan solutions. **A**, Frequency sweep plot of the elastic (G') and viscous (G'') modulus for normal SF and for SF from osteoarthritic joints⁵⁵. The cross-over frequency typically moves to higher values as solutions become more dilute and/or MW decreases. **B**, Fraction of injected HA diffusing from SF into the tissues as a function of MW⁶².

In this respect, an interesting insight was provided by data on trans-synovial flow^{61,62}. The trans-synovial flow is a very well-regulated process in the joint, since even a small, sustained imbalance of it would quickly lead to joint swelling or fluid depletion. Sustained flexion is a particular threat to volume homeostasis, because it raises intraarticular pressure, driving fluid out of the joint cavity. An important function of endogenous HA in the synovial fluid is to counter this threat by “buffering” the fluid drainage rate. Coleman et al⁶¹ showed that the outflow buffering effect is dependent on the HA molecular weight. In this study HA of different MW were infused into the knees of anesthetized rabbits, with Ringer solution as control in the contralateral joint, and trans-synovial drainage rate was recorded at known joint pressures. With HA in the MW range here considered the fluid drainage rate was reduced relative to Ringer solution, but increased with pressure, indicating that there was no full outflow buffering. In particular, data showed (**Figure 1B**) that a significant amount of the administered HA was able to diffuse into the articular tissues⁶² and, as a consequence, to interact with cells and receptors, a result consistent with previous studies performed *in vivo* with fluoresceinated HA^{63,64} or with ¹⁴C-labeled HA⁶⁵ that demonstrated how administered HA in this MW range was present at the level of the pericellular matrix of synoviocytes and chondrocytes after a single intra-articular injection with reported concentrations of about 200 µg/g in the synovial tissue and 25 µg/g in the articular cartilage. This finding opened the possibility that 500-730 kDa HA could mainly act through some biological mechanism of action. Studies undertaken to test this hypothesis will be briefly reviewed in the next section.

in vitro and in vivo Preclinical Studies

The quite large amount of available *in vitro* and *in vivo* studies carried out with 500-730 kDa HA supports the idea that this HA preparation can exert a combination of modulating biological activities on the cell populations present in the joint by acting on specific receptors (CD44, ICAM-1, RHAMM, LYVE-1) expressed on the surface of various cell types, including inflammatory cells, chondrocytes and synoviocytes⁶⁶⁻⁶⁹.

Effects on Inflammatory Cells and Modulation of Inflammation

Early *in vitro* studies⁷⁰ have shown that HA in the MW range here considered inhibited, in a dose dependent manner, the migration, che-

motaxis and adhesion to substrata of leukocytes at concentrations (0.5-1.5 mg/ml) that can be obtained following the standard treatment schedule. The same effect was not observed with other charged polysaccharides, such as dextran sulphate or chondroitin sulphate. Further experiments⁷¹ indicated that HA was able to alter cell locomotion in at least three ways, namely inhibition of chemotactic gradient formation, prevention of binding of chemoattractant to cells, and direct inhibition of cell adhesion. In this respect, subsequent *in vivo* researches⁷² showed that HA receptors were deeply involved in the regulation of leukocyte locomotion, since blocking or deleting CD44 resulted in a decrease of the cell recruitment into the synovial fluid and in a reduction of the severity of the experimentally induced arthritis in mice.

The influence of 500-730 kDa HA on the growth of human macrophages was explored by an *in vitro* study⁷³. The results showed that at a dose of 1mg/ml, HA significantly reduced the rate of cellular proliferation and altered cell cycle distribution. Concomitantly, a 10-fold increase in apoptotic cells and a 12-fold increase in dead cells were observed after 168 hours. Additional data from this work also suggested that the observed effect was likely mediated by the interaction of HA with the cell surface via CD44 receptors.

Recently, evidence was provided⁷⁴ that platelets may contribute to joint degeneration in OA by favoring the accumulation of matrix metalloproteinase 2 (MMP-2) in the SF. In fact, when fibroblast like synoviocytes (FLS) isolated from SF of OA patients were co-cultured with platelets the release of MMP-2 was favored by the interaction of platelet surface P-selectin with FLS CD44. In the presence of 500-730 kDa HA the increase of MMP-2 production by FLS, triggered by the interaction with platelets, was significantly reduced in a dose-dependent manner.

These findings show consistency with animal studies indicating that this specific HA preparation modulated both acute and chronic inflammation in a dose related fashion⁷⁵, and with immunological and biochemical evaluations obtained as part of human studies. In a cytological and cytofluorimetric study focused on the inflammatory cell populations present in the synovial fluid of patients with OA and joint effusion following treatment with Hyalgan^{®76} not only a decrease in cellularity of the synovial fluid (leukocytes, monocytes, lymphocytes) was observed, but also a significant reduction in activated lymphocytes

and monocyte-macrophage phenotypes after HA treatment, when compared with a placebo-treated group. Placebo-controlled studies estimating the production of inflammatory mediators and catabolic factors were performed by Punzi and coworkers^{77,78}. They indicated that 500-730 kDa HA injections were able to reduce prostaglandin E₂ (PGE₂) and MMP (MMP-1 and MMP-3) levels in knee SF of patients with various arthropathies. A significant reduction of the total amount of arachidonic acid metabolites and cytokines (LTC₄, PGF_{1α}, PGF_{2α} and interleukin-1β) in human SF following treatment with HA in the MW range here considered was observed by Hirota et al⁷⁹.

Altogether these results provided indication that the beneficial effects of 500-730 kDa HA intra-articular therapy could be in part linked to its ability to control the inflammatory process by a downregulation of the inflammatory cells activity.

Effects on Tenocytes

Osti et al⁸⁰ *in vitro* recently provided evidence of a possible effect of hyaluronan on viability, metabolic activity, apoptosis, and collagen type I expression in human tendon-derived cells. The results indicated that following hyaluronan administration cell viability and proliferation increased in a dose dependent manner. Furthermore, HA stimulated the synthesis of collagen type I in a dose dependent fashion, without increase in collagen type III. No dependence of the effect on the MW of the administered hyaluronan was observed in this *in vitro* study. As far as 500-730 kDa HA is concerned, its effect on tenocytes was also tested *in vivo* by Salamanna et al⁸¹. In this study, the right patellar tendon of rats that underwent discontinuing training activity received repeated peri-patellar injections of either 500-730 kDa HA or saline, while the left tendon was untreated. Cells derived from the tendons were then cultured and tenocyte morphology, metabolism and synthesis of C-terminal-propeptide of type I collagen, collagen-III, fibronectin, aggrecan, tenascin-c, interleukin-1β, MMP-1 and MMP-3 were evaluated after 1, 3, 7 and 10 days of culture. Cultures from HA-treated tendons showed a significantly higher proliferation rate and viability, and increased synthesis of C-terminal-propeptide of type I collagen, fibronectin, aggrecan, tenascin-c and matrix-metalloproteinase-3 with respect to the saline-treated ones, whereas synthesis of MMP-1 and interleukin-1β was decreased. The results, therefore, suggested that 500-730 kDa HA can allow the maintenance

of tenocyte anabolic activity, a finding of potential relevance to enhance a positive response of the tendons to pathological insults.

Effects on Chondrocytes

An early study on an animal model of osteoarthritis showed that 500-730 kDa HA treatment induced a beneficial effect on the cartilage response to damage, as assessed by morphological and morphometrical analysis of the tissue⁸². Similar results were subsequently obtained both in dogs⁸³ and in rabbits⁸⁴ suggesting that sequential cycles of 500-730 kDa HA therapy may provide long-term benefits for altering the disease course.

in vitro studies highlighted a variety of biological effects of this HA fraction on chondrocytes. In chondrocyte cultures, indeed, 500-730 kDa HA was shown to guard these cells against nitric oxide⁸⁵ since it was able to reduce, in a dose-dependent way, the synthesis of both interleukin-1-induced nitric oxide and PGE₂. Of note, the effect was not evident with hyaluronic acid of higher molecular weight (6000 kDa). It must be observed that nitric oxide and PGE₂ are among the most potent mediators of cartilage damage and nitric oxide is also directly involved in cell apoptosis. In this respect, Grishko et al⁸⁶ evaluated the chondroprotective action of this HA preparation on cultured human articular chondrocytes following experimental stress induced by reactive oxygen (ROS) or nitrogen (RNS) species, mimicking increased nitric oxide (NO) and RNS production during OA progression. 500-730 kDa HA caused a decrease in mitochondrial DNA damage, enhanced mitochondrial DNA repair capacity, cell viability and decreased apoptosis demonstrating that enhanced chondrocyte survival and improved mitochondrial function under conditions of oxidative injury are important components of the therapeutic action of Hyalgan® in osteoarthritis.

This HA preparation was also shown to protect chondrocytes against anti-Fas induced apoptosis *in vitro*⁸⁷. In this cellular model, blocking the CD44 and ICAM-1 HA receptors, the protective effect disappeared, demonstrating that it was receptor mediated. Protection against apoptosis during the development of OA was also observed *in vivo* in rabbits following treatment with HA in the MW range here considered⁸⁸.

The inhibition of the catabolic activity of chondrocytes is a further effect induced by 500-730 kDa HA administration to the cells, as demonstrated by a study⁸⁹ monitoring the release of proteoglycans from the cell matrix fraction into the medium in

chondrocyte cultures, a parameter considered as a good index of the catabolic activity of the chondrocytes⁹⁰. In that study, rabbit chondrocytes were cultured in the presence of cytokines (interleukin-1 β , tumor necrosis factor α) and HA with MW between 300 and 800 kDa was found to be a potent inhibitor of the release of proteoglycans. The effect was dose dependent in a range of concentrations (10-1000 μ g/ml) comparable to those obtainable *in vivo* in the tissue following intra-articular administration of the substance.

An enhancement of the anabolic activity of chondrocytes was also demonstrated following 500-730 kDa HA administration. Chondrocytes regulate the cartilage homeostasis by secreting various substances. In this respect, the balance between the activity of MMP and their inhibitor (TIMP-1) is thought to be important for the maintenance of cartilage matrix within articular tissues. The MMP/TIMP ratio is therefore an index of cartilage degradation and it has been demonstrated that HA in the MW range here considered is able to reduce such a ratio in chondrocytes cultured for 8 days in presence of interleukin-1 β ⁹¹. In cultured rabbit chondrocytes⁹² and in bovine articular cartilage⁶³ this HA fraction appeared also able to enhance proliferation and matrix synthesis that was reduced by interleukin-1 β . These findings are also supported by the *in vitro* results on normal and osteoarthritic cartilage explants demonstrating that 1mg/ml HA (in the MW range here considered) was able to block interleukin-1 β stimulated production of MMP-1, MMP-3 and MMP-13 in normal and osteoarthritic cartilage⁹³.

The CD44 receptor is likely involved in the modulation of the anabolic activity of chondrocytes, as demonstrated by *in vitro* studies^{67,93,94}. The adhesion of chondrocytes to HA through CD44 receptor, for instance, induced a variety of stimulatory signals, such as c-myc mRNA and transforming growth factor- β mRNA expression, leading to maturation or differentiation of chondrocytes and regulating chondrocyte proliferation as well as matrix synthesis in the cartilage microenvironment⁶⁷. Furthermore, the stimulatory effect of 500-730 kDa HA on chondrocyte proliferation and survival was no longer present when chondrocytes were cultured in the presence of CD44 antibodies⁹⁴.

Effects on Synoviocytes

An effect of great interest triggered by 500-730 kDa HA was identified by Smith and Ghosh⁴⁵ in synoviocytes. In this early *in vitro* study, the authors showed that synovial fibroblasts obtained

from knee joints of patients with OA synthesized HA at a lower rate than cells derived from normal synovia. They, however, responded to the presence of HA in the MW range here considered by increasing the biosynthesis of HA in a concentration dependent way and at concentrations (50-100 μ g/ml) comparable to those obtainable in the tissue following intra-articular administration. This process is of particular relevance, because once initiated it appeared self-sustaining. Thus, it might explain the prolonged duration of the effect following intra-articular administration. The synthesis of high MW endogenous HA by synoviocytes as a consequence of 500-730 kDa HA administration was subsequently confirmed *in vivo* in an ovine model of OA⁹⁵. In this study the intra-articular administration of Hyalgan[®] resulted in a significant 70% increase of the endogenous hyaluronic acid in the SF. Interestingly, the increase was low and not significant when the animals were treated with HA of higher MW (2300 kDa), consistently with the lower accessibility to synoviocytes of high molecular weight HA.

The role of CD44 receptor in the modulation by HA of the biosynthetic mechanisms in synovial fibroblasts was evidenced by a study on synoviocytes of patients suffering from rheumatoid arthritis⁶⁸. In this study, HA binding to CD44 up-regulated the mRNA transcription and the expression of VCAM-1. Such a cross-talking between adhesion molecules might be of importance in the regulation of the inflammatory process.

Interaction between HA and HA-Receptors

As illustrated in the previous sections, many biological activities triggered by 500-730 kDa HA were found to be mediated by its interaction with specific HA receptors at the cell membrane, in particular CD44 (see^{67,68,72,73,87,93,94}). In general, the consequence of the interaction between HA and HA receptors is to stimulate transduction and other signaling pathways that modulate cell functional activities manifested primarily by cell migration, proliferation, endocytosis, and changes in synthetic activity^{69,96}. Studies⁹⁷⁻¹⁰⁰ on the effect of HA on CD44 cellular signaling, however, have shown that the results depend on the size of the HA molecules used. For instance, 200 kDa HA was shown to be more effective in maintaining the survival of blood eosinophils than HA with an MW of 3×10^6 Da¹⁰¹. The authors proposed that this protective effect was mediated via the increased expression of granulocyte mac-

rophage colony-stimulating factor after CD44 activation. Further support to these findings were quite recently provided by Rayahin et al¹⁰² who showed that macrophages undergo phenotypic changes dependent on HA molecular weight. In the abovementioned study by Smith and Ghosh⁴⁵, investigators found the most marked response by synovial fibroblasts from an osteoarthritic joint exposed to HA of MW greater than 500 kDa, whereas smaller molecules had little or no effect. Moreover, a high-MW hyaluronan (4700 kDa) was less effective than a preparation of 3800 kDa when tested in the same experimental model.

Molecular studies have shown that hyaluronan binding at the cell surface is a complex interplay of CD44 receptor density, CD44 activation state and multivalent binding events affected by the size of the hyaluronan ligand¹⁰³. The molecular mechanism responsible for the MW-dependent action of HA on its receptors, indeed, may be at least in part related to the clustering and cross-linking of CD44 on the plasma membrane inducing a reorganization of the cytoskeleton proteins^{100,104,105}. It is known that binding of HA to the CD44 link module uses discrete exasaccharide elements along the glycosaminoglycan chain¹⁰⁶. Thus, oligomers of proper size can interact with more than one CD44 receptor (Figure 2), and the cellular response ensued from HA binding will depend on the formed pattern of receptor complexes^{106,107}. Beyond a certain limit, however, very large hyaluronan molecules may become less efficient in engaging multiple receptors because of steric hindrance⁹⁹. Therefore, it can be suggested that the maximal response from a given cell type would be produced by hyaluronan within a specific size range (neither too big nor too small).

The situation is further complicated by the existence of different isoforms of the CD44 receptor¹⁰⁸, and the observation that HA can readily enter cells by an unusual endocytic route and interact with specific intracellular proteins¹⁰⁹. Several intracellular HA binding proteins (receptors) have been described^{96,107}. One of these, RHAMM (receptor for HA mediating motility) is normally associated with the actin cytoskeleton and microtubules, where it colocalizes with erk 1 protein and MEK1 and modulates their signaling. Significantly, the binding domain for erk 1 on RHAMM is the same as for HA, suggesting the possibility that HA of suitable size to reach the cells in the tissues may compete for the erk 1 binding site within the cell⁹⁶.

Based on these features of the HA-CD44 interaction, Ghosh and Guidolin⁹⁷ suggested that

the mechanism of action of intra-articular HA was dependent on the MW. Since the HA present in normal joint tissues is generally of high MW, it would seem to make biological sense for it not to continuously stimulate an active response from the cells it surrounds. On the contrary, a mechanism of action which is mainly of pharmacological type (i.e. receptor-mediated) can be surmised for administered HA in the mid-MW range, such as 500-730 kDa HA. In fact, HA of this size can significantly diffuse into the tissues and reach the cells (see section "Early evidence") where it could provoke a pattern of CD44 clustering and crosslinking on binding suitable to trigger a cellular response. For what it concerns the joint tissues, the main experimental support to this concept were the abovementioned studies by Ghosh and coworkers^{45,95}, showing that human synovial fibroblasts derived from OA joints when cultured with HA responded by up-regulating or down-regulating endogenous HA synthesis, depending on the media concentration and the MW of the exogenous HA added. The maximal stimulation of endogenous HA synthesis was produced by HA with MW around 0.5×10^6 Da, and the cell response significantly decreased when HA of MW of about 3×10^6 Da were used. The term *viscoinduction*⁹⁷ was then coined to describe the main mechanism of action exploited by HA of MW between 0.5×10^6 – 1×10^6 Da to induce clinical benefits following intra-articular administration, whereas the *viscosupplementation* concept appears more appropriate to describe the physical mechanism of action mainly exploited by high-MW HA and by products based on modifications of HA molecules to achieve greater elastoviscosity and intra-articular dwell-time^{110,111}.

The biological properties of HA in the MW range here considered may therefore allow to overcome the questions raised by early studies, based on the rheology of this material in comparison to healthy SF (see section "Early evidence"). Furthermore, they suggest that significant differences could exist in terms of mechanism of action among the various HA-based formulations proposed for the intra-articular therapy of OA^{99,112}. In this respect, however, a second question can be raised. It is based on the well-known depolymerization processes affecting the administered material as a consequence of the presence of oxygen-derived free radicals, hyaluronidases, and other glycosidases in the joint environment. At least from a theoretical standpoint, starting from very high-MW HA these processes could

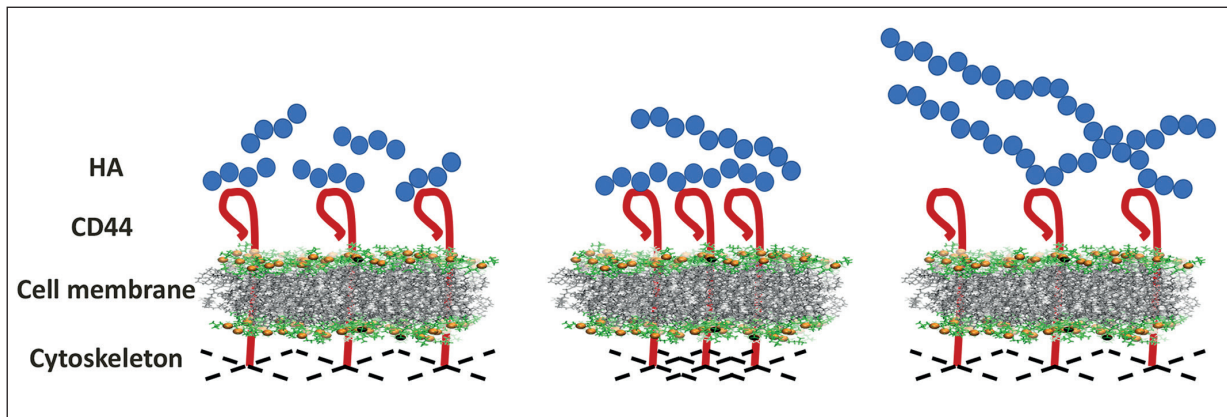


Figure 2. Schematic view of the model derived from the studies by Smith and Ghosh⁴⁵ for the binding of HA to CD44 receptors as a function of MW⁹⁹. HA of small MW (left panel) can bind only monomers of the receptor without inducing any receptor clustering. When HA size exceeds a given threshold (central panel), each chain can interact with more than one receptor inducing receptor clustering and a reorganization of the cytoskeletal proteins, which in turn triggers a cell response. Beyond a certain limit (right panel), however, very large HA chains may become less efficient in engaging multiple receptors because of steric hindrance.

generate sufficient amount of HA of the suitable size to trigger a therapeutically positive biological response. Thus, the difference between HA of different size in terms of mechanism of action would be more apparent than real. Data on this topic were provided by Komatsu et al¹¹³ in their study on the kinetics, metabolism and reutilization of HA after intra-articular administration. An interesting finding of this study concerned the pattern of degradation products generated starting from injected high-MW HA (2·10⁶ Da). In fact, fragments with MW lower than the MW range here considered (of 300 kDa at first and, at a later stage, of 50 kDa) were mainly found in the articular environment. They were then broken down by cells into C units (carbon cycle) before being re-used as an *in vivo* constituent of the body. These results, therefore, provided support to the idea of a substantial difference in terms of mechanism of action between high- ('viscosupplementation') and mid-MW ('viscoinduction') HA preparations for intra-articular therapy.

Clinical Investigations

A quite large number of clinical investigations were focused on 500-730 kDa HA as a therapeutic tool in joint pathology^{46,49,50,51,114-171}. As schematically illustrated in Table II, they encompass studies (involving a quite high number of patients) that addressed pathological conditions of different joints, the clear majority being focused on OA.

The most common treatment schedule consisted of intra-articular injections of 1% (wt/vol)

HA solution once a week for 3-5 weeks. Different regimens were also tested^{144,151}, in particular to address hip and shoulder conditions. Some of these studies reported lack of efficacy of the treatment^{125,152,155}, which resulted comparable to placebo. Overall, however, data from open-label and placebo-controlled studies provided support to the early findings, demonstrating significant symptomatic and functional improvement in patients with OA. In a study on 108 patients¹¹⁷, for instance, the mean percentage of pain reduction (as estimated by VAS following exercise) was 60% at the end of the treatment, 67% after 6 months and 72% after 12 months. Almost all patients who completed the 12 months follow-up reported an improvement in their pain condition (93% of patients at the end of treatment and 97% at month 12). Along the same lines, are further studies^{118,120,123,131,139,144,148} reporting not only relief of pain following treatment, but also improvement of functional performance, as evaluated by Lequesne's scale¹³⁹, WOMAC score¹¹⁸, or 50-foot walking test¹³⁰. A recent meta-analysis¹⁴⁰ showed that a three-weeks course of 500-730 kDa HA treatment was sufficient to obtain the symptomatic effect, but repeated cycles of treatment were reported to provide additional symptomatic benefit^{114,115,119,143}.

Comparative studies indicated that 500-730 kDa HA was similar in efficacy to reference therapeutic approaches, such as oral NSAID, or corticosteroid injections^{133,134,137}, and superior to other intra-articular treatments, such as orgo-tein or glycosaminoglycan polysulphate¹⁶⁶⁻¹⁶⁸, in

Table II. Clinical studies on intra-articular 500-730 kDa HA.

Joint	Design	Reference	Therapeutical application	No. of patients
Knee	Open label	Scali ¹¹⁴	OA	75
		Kotz and Kolarz ¹¹⁵	OA	108
		Neustadt ¹¹⁶	OA	76
		Kolarz et al ¹¹⁷	OA	108
		Birchall et al ¹¹⁸	OA	100
		Turajane et al ¹¹⁹	OA	183
		Phiphobmongkol et al ¹²⁰	OA	31
		Miller and Block ^{121,122}	OA	553
		Mahesh et al ¹²³	OA	45
	Placebo-controlled	Miller et al ¹²⁴	OA	218
		Dixon et al ⁴⁶	OA	63
		Dougados et al ⁵⁰	OA	110
		Henderson et al ¹²⁵	OA	91
		Carrabba et al ⁵¹	OA	100
		Listrat et al ¹²⁶	OA	39
		Frizziero et al ¹²⁷	OA	40
		Bunyaratavej et al ¹²⁸	OA	49
		Jubb et al ¹²⁹	OA	408
		Huang et al ¹³⁰	OA	200
		Khalaj et al ¹³¹	OA	25
		Ip and Fu ¹³²	Arthritis	70
	Parallel-design	Jones et al ¹³³	OA	63
		Altman et al ¹³⁴	OA	495
		Pasquali Ronchetti et al ¹³⁵	OA	99
		Guidolin et al ¹³⁶	OA	24
		Frizziero and Pasquali Ronchetti ¹³⁷	OA	148
		Forster and Straw ¹³⁸	OA	38
		Huang et al ¹³⁹	OA	140
		Stitik et al ¹⁴⁰	OA	60
		Raman et al ¹⁴¹	OA	392
		Westrich et al ¹⁴²	Arthroscopy	50
		Barenbaum et al ⁴⁹	OA	437
		Chareancholvanich et al ¹⁴³	Osteotomy	40
Shoulder	Open label	Leardini et al ¹⁴⁴	Painful shoulder	29
		Meloni et al ¹⁴⁵	Tendinosis	56
		Bernetti et al ¹⁴⁶	Lateral epicondylalgia	11
	Placebo-controlled	Blaine et al ¹⁴⁷	Painful shoulder	456
		Tagliafico et al ¹⁴⁸	Cuff tear arthropathy	90
	Parallel-design	Di Giacomo and De Gasperis ¹⁴⁹	OA	61
Hip	Open label	Bragantini and Molinaroli ¹⁵⁰	OA	44
		Migliore et al ¹⁵¹	OA	14
	Parallel-design	Qvistgaard et al ¹⁵²	OA	101
Ankle	Open label	Sun et al ¹⁵³	OA	46
	Placebo-controlled	Salk et al ¹⁵⁴	OA	20
		Cohen et al ¹⁵⁵	OA	30
	Parallel-design	Sun et al ¹⁵⁶	OA	75
TMJ	Open label	Guarda-Nardini et al ¹⁵⁷	OA	25
		Yakan and Toameh ¹⁵⁸	OA	20
	Parallel-design	Guarda-Nardini et al ¹⁵⁹	OA	90
		Gorrela et al ¹⁶⁰	Functional disorders	62
		Kiliç and Güngörmüş ¹⁶¹	OA	31
Hand	Open label	Schumacher et al ¹⁶²	OA	16
		Klauser et al ¹⁶³	OA	33
		Frizziero et al ¹⁶⁴	OA	58
	Placebo-controlled	Locati et al ¹⁶⁵	OA	20

reducing pain and improving functional scores.

The 26-week, double-blind, placebo- and Naproxen-controlled, multicenter trial in the U.S., coordinated by Altman and Moskowitz¹³⁴ provides an example. The results of this study, involving 495 patients with OA of the knee, indicated that 500-730 kDa HA therapy (5 injections, once a week) was significantly more effective than placebo and at least as effective as continuous Naproxen (500 mg twice a day orally for 26 weeks) in terms of relief of pain as assessed by VAS during a 50-foot walk test, and by a categorical scale. The treatment also improved joint function. At the end of the follow-up (26 weeks), HA treatment was superior to placebo, and comparable to Naproxen, in the secondary outcome parameters such as the WOMAC scale, heel-to-buttock distance, and knee range of motion.

The early observation that the induced effects were long lasting was also essentially confirmed by subsequent studies considering a follow-up of one^{117,118,145,151,157} or two years¹¹⁶ following a single cycle of therapy. In this respect, of particular interest could be recent studies focused on the use of combined HA and physical therapy^{121,122,124,132,140,156}. As reported by Miller et al^{121,122,124}, patients with knee OA were enrolled in an 8-week multimodal treatment program, involving a cycle of five weekly intra-articular injections of HA followed by a structured physical therapy and education programs provided by physical therapists 2-3 times per week during the 8-week period. Interestingly, long-term follow-up data (mean 3.7 years) were obtained from 218 patients¹²⁴ and showed that WOMAC scores were still significantly lower at the long-term follow-up when compared to baseline.

Clinical studies on intra-articular 500-730 kDa HA therapy, however, are of interest for the present discussion not only because of the reported data on the symptomatic efficacy of this type of treatment but also in view of provided additional information that may allow a better characterization of the specific features of this HA preparation and, by comparison, of other HA-based tools. Thus, this quite documented clinical experience may suggest criteria to better position the various available HA-based preparations in the panel of the therapeutic strategies for the management of joint disease. According to this standpoint, therefore, some details emerging from the clinical studies on 500-730 kDa HA will be briefly discussed in the sections that follow.

Therapeutic Indications

A first question that can be raised concerns the profile of OA patients candidate to obtain the highest benefit from the therapy. Although no studies specifically designed to address this question are available⁹⁹, data from some investigation suggested that the intra-articular treatment with 500-730 kDa HA performed best in patients with mild-to-moderate disease. In a pilot clinical evaluation of the treatment of hip osteoarthritis¹⁵⁰ forty-nine joints were treated: thirty-four had mild-to-moderate OA¹⁷² and fifteen had severe OA. When the patients were stratified by roentgenographic severity of the disease, those with mild-to-moderate OA showed the greatest improvement for all the parameters tested. More recently, consistent findings were reported by Turajane et al¹⁶⁹, who showed that the treatment was effective in visible cartilage patients (Ahlback grade 1) without mechanical problems, while in severe OA patients (Ahlback grade 3,4,5) the treatment was of less benefit. The clinical scenario of patients with mild-moderate OA, clinically and radiologically assessed, who have not received other therapies was also confirmed as “appropriate” for this type of treatment in a meta-analysis very recently proposed by a workgroup of clinical experts who developed an “Appropriate Use Criteria” for HA¹⁷³. In this context, of interest are also some data obtained in trials comparing 500-730 kDa HA and Hylan G-F20, a high-MW cross-linked HA-based preparation. Raman et al¹⁴¹, for instance, reported that in patients with a more severe OA and minimum pain score of 6 on a VAS scale the symptomatic benefit was higher and more sustained following Hylan treatment, further suggesting the possibility that in distinct patient populations HA differing in MW could have a different efficacy profile. However, since solid evidence has not been provided so far²³, additional head-to-head studies should be considered to establish this point⁹⁹.

If demonstrated, this hypothesis would show consistency with the above discussed possible dependence on the MW of the HA mechanism of action. It can be surmised¹⁷⁴, indeed, that a HA-based preparation able to mainly trigger positive biological responses from joint tissues has likely more potential to target the condition of patients with mild-to-moderate disease and a low grade of joint tissue degeneration, where some tissue response and possibly repair can still be stimulated. On the other side, when the disease progresses to higher levels of tissue degeneration HA-based

preparations able to obtain a more efficient viscoelastic, mechanical, support may become more effective. Studies demonstrating that in patients undergoing arthroscopic surgery the intra-articular administration of 500-730 kDa HA can lead to more pain relief and functional mobility than after arthroscopy alone^{142,143,160}, as well as studies on joint pathologies other than OA^{145,146,170,171}, provided indirect support to this view.

Disease Modifying Activity

Consistent with a mechanism of action for 500-730 kDa HA mainly of biological type are clinical data indicating that this treatment can have beneficial effects on structural parameters of the joint tissues, opening the possibility of a delay in the progression of the cartilage breakdown in patients suffering from OA.

The first study on this topic was carried out by Listrat et al¹²⁶. A total of 39 patients were enrolled in this randomized, controlled, prospective, pilot clinical study on knee OA. The treated group received three cycles of three intra-articular injections of Hyalgan® at three-month intervals between each course of injections plus joint lavage whereas the control group was treated with intra-articular joint lavage alone. The degree of chondropathy in the medial compartment of the knee was assessed by SFA (Société Française d'Arthroscopie) scoring systems. After one year the authors reported a statistically significant slowing-down of the progression of cartilage lesions in the HA-treated group as compared to the control one.

Subsequently, 408 patients with primary knee OA were recruited by Jubb et al¹²⁹ for a randomized, double-blind, masked-observer, placebo-controlled study aimed at investigating the structure-modifying activity of 500-730 kDa HA treatment by using X-ray evaluation of the joint space narrowing (JSN) in the medial tibial-femoral compartment of the knee. The primary endpoint (JSN) was in accordance with the recommendations of the CPMP (CPMP/EWP/784/97) for structure-modifying medicinal products for the treatment of osteoarthritis, and patients received three cycles of three weekly intra-articular injections of HA or placebo (saline) at 4-months intervals. At one-year timepoint JSN was measured using digital image analysis of standardized radiographs, and the results showed that, when compared to the placebo group, HA treatment was able to slow-down the progression of OA of the knee in patients with radiologically

less severe disease at baseline. More recent studies provided further support to these findings, showing that intra-articular 500-730 kDa HA injections may be beneficial for preventing articular cartilage degeneration¹⁴⁵ and increasing total cartilage volume¹⁴³.

In this context, of interest are also studies involving the analysis of synovial membrane and cartilage biopsies obtained during arthroscopic investigation^{127,137}. The alteration in synovial membrane histopathology assessed using electron microscopic evaluation of biopsies was the primary outcome of a study conducted in 99 patients with either primary or secondary OA of the knee and comparing the effects of five intra-articular injections of 500-730 kDa HA with those of three injections of methylprednisolone acetate¹³⁵. Both active treatments significantly decreased inflammation and produced favorable modifications in several structural aspects of the synovial membrane. Edema was decreased, and the amount of collagen present in the membranes was increased. HA, but not methylprednisolone, also significantly reduced the number of aggregated synoviocytes. When cartilage biopsies taken from a subset of 24 patients with primary OA, were analyzed¹³⁶, the results indicated that HA significantly improved chondrocyte density and overall matrix appearance, compared with methylprednisolone treatment. Furthermore, the authors showed that 500-730 kDa HA treatment was able to change chondrocyte metabolism from predominantly catabolic to predominantly anabolic, as indicated by the increased extension of the synthetic structures and mitochondria with respect to the organelles having catabolic or storage functions.

Taken together, available clinical results form a quite solid body of evidence in support of the ability of 500-730 kDa HA to modify OA disease progression and are consistent with preclinical data. It is noteworthy that these clinical studies made use of the OARSI recommended guidelines for the design of studies aimed at demonstrating disease modification: the primary outcomes were based on prospective evaluations of either imaged or directly visualized measures of joint structure and morphology and outcomes were often evaluated at the recommended timepoint of 1-year¹⁷⁵. One important issue that requires further information is the possible impact of HA MW on disease-modifying activities, since current preclinical data suggest that MW may be an important factor (see^{87,95,101}). Unfortunately,

clinical data supporting disease modification are essentially limited to the HA fraction here considered and comparative trials evaluating structural outcomes of treatment with HA products of different size and manufacture would be needed to further investigate this issue in patients.

Safety

As a class, the HA-based preparations have a well-documented high tolerability profile with no known systemic effects and few contraindications or drug interactions¹⁷⁶. From studies involving native HA preparations an incidence of adverse events of 0.5-0.8% has been estimated¹⁷⁴, mostly of minor clinical significance, the common adverse event reported being injection site pain. With the use of chemically modified HA-preparations (engineered to achieve greater viscoelastic properties and higher dwell-time) Goldberg and Coutts¹⁷⁷ report an incidence of 8-27% of acute local reactions. In particular, a clinically distinct reaction known as pseudosepsis or SAIR (severe acute inflammatory reaction) has been often associated to hylans⁹⁹. This observation likely depends on the type of contaminants generated during cross-linking that have been shown to be immunologically distinct from those present in native HA preparations^{176,178}. Rabbit studies, indeed, demonstrated an inflammatory reaction to hylan but not to native 500-730 kDa HA after injection in the joint space⁴⁷. Interestingly, reports exist of patients having a SAIR and subsequently being treated with native HA with good clinical results¹⁷⁷, further confirming the possibility of differences in the safety profiles of different HA-based products.

Cost Analysis

A point of potential difference between HA-based tools for intra-articular therapy can be their impact on disease-specific costs. The relevance of the topic was evidenced by research showing that the annual estimated number of people in the USA with OA was approximately 30.8 million for 2008-2011¹⁷⁹. When pathologies of the knee are considered, about 644,000 total knee replacement (TKR) surgeries were performed in 2011, 97% of which were due to OA¹⁸⁰. TKR is highly effective in patients with bone-on-bone OA and significant knee symptoms, with durable symptom reduction in 80-90% of cases¹⁸¹. However, widespread adoption of TKR is hindered because of high expense, unacceptable complication risk, and lack of perceived benefit^{182,183}. Thus, cost-effective

alternative treatments with better patient acceptance are of key importance.

Studies addressing the impact of 500-730 kDa HA therapy on the need of TKR were proposed by Turajane et al^{119,169}. The authors performed a retrospective analysis of the data in their hospital focused on the years 2001-2004 and 183 patients were enrolled. All patients received repeated cycles of intra-articular HA. After 54 months the reported incidence of TKR was 28.4% with a mean time to TKR of 15.4 months. The estimated cost savings for cancellation or delayed surgical procedures was estimated to be about 63%. The effect of an 8-weeks multimodal treatment involving a single cycle of intra-articular injections of 500-730 kDa HA followed by a physical therapy and education program was studied by Miller et al¹²². A total of 553 patients were contacted at 1 year ($n = 336$) or 2 years ($n = 217$) follow-up. The percentage of patients who underwent knee arthroplasty was 10% at 1 year and 18% at 2 years following program completion. The treatment program resulted highly cost effective at \$12,800 per quality-adjusted life year at 2 years, and cost effectiveness was maintained under a variety of plausible assumptions and regardless of gender, age, body mass index, disease severity, or knee pain severity. Consistent results were reported by Ip and Fu¹³² following a combined therapy involving HA injections and low-level laser irradiation. A cost-effectiveness analysis was also provided for a high molecular weight, bioengineered HA, based on data obtained from a clinical trial¹⁸⁴, concluding that the HA product was less costly and more effective than conventional care with NSAID and analgesics.

A comparison across different HA-based products was recently proposed by Dasa et al¹⁸⁵ and is of particular interest for the present discussion. The study compared different US FDA-approved HA viscosupplements (including Hyalgan®) using real-world evidence from IMS Health's PharMetrics Plus Health Plan Claims Database, which comprises adjudicated claims for more than 150 million unique patients across the USA and has diverse representation of geography, employers, and payers. The primary outcome measures were disease-specific costs associated with knee OA and time from the index date to TKR surgery. 50,389 patients with HA treatment for knee OA were identified. 18,217 (36.2%) patients were treated with HA products indicated for five injections per treatment course. The remainder were treated with HA products indicated for fewer than

five injections per treatment course, with 20,518 patients (40.7%) receiving chemically modified HA-based preparations (Hylan). Hazard ratios showed a significantly higher risk of TKR for patients receiving chemically modified HA compared to native HA-based products. Consistently, patients treated with native HA had longer delays to TKR than those treated with Hylan. Although this study did not show any clear relationship between molecular weight of HA and its ability to delay TKR, nevertheless this analysis of administrative claims data provides real-world evidence that meaningful differences exist among HA products in terms of disease-specific cost and time to knee replacement surgery.

Conclusions

Intra-articular HA injections for the symptomatic relief of pain have been available for treatment since the 1980s. Practitioner experience¹⁸⁶ and real-world evidence¹⁸⁵ have been accumulated to suggest that HA injections are effective and well tolerated in patients who either do not respond adequately to conventional therapy or who are intolerant of nonsteroidal anti-inflammatory drugs¹⁸⁷. Treatment guidelines issued by different professional medical societies, however, do not point in a single direction, most of them accepting this treatment modality, some not recommending its use. This appears mainly due to conflicting results of the proposed meta-analyses³⁶ at least in part associated with a marked variability between different HA preparations on different outcome parameters, as often reported by analysts^{17,22}. A recent meta-analysis from Altman et al⁴² provided support to this concept, suggesting that intra-articular HA products should not be treated as a group, as there are differences between them that influence both efficacy and safety.

From a biological standpoint it must be observed that injected HA can trigger a variety of responses including effects on joint mechanics and nociception, effects on inflammation, and effects on cell metabolism¹¹², depending on the MW, viscoelastic characteristics and accessibility to joint tissues. Thus, each available HA-based preparation for intra-articular therapy is likely characterized by a specific profile as a therapeutic tool. The focus of the present review article was a particular HA preparation (500-730 kDa HA, Hyalgan®), since its use dated back to thirty years ago and a quite large amount of biophysical, preclinical and clinical stud-

ies are available documenting its characteristics. The rheological profile and the half-life of injected 500-730 kDa HA does not support a mechanical role for this material^{55,56,110}. Its biophysical characteristics, however, can allow a significant diffusion into the joint tissues⁶² to interact with cells and receptors. Interestingly, the reported elastoviscosity of synovial fluid one week after administration of the treatment (well after the product was cleared from the joint space) resulted increased relative to pretreatment values⁵⁵, a finding consistent with the effects on synoviocytes demonstrated in cell cultures and animal models of OA^{45,95}. Thus, a mechanism of action mainly of pharmacological type (i.e. receptor-mediated) is likely at the basis of the therapeutic outcomes of the treatment. In particular, it supports the long-term effects observed in several clinical studies showing long-lasting symptomatic benefit following treatment, a result that can be further increased by combining HA injections and physical therapy¹²²⁻¹²⁴. In this respect, of particular interest is also the body of evidence¹⁷⁵ supporting the ability of 500-730 kDa HA to counteract cartilage degradation and the OA disease progression by modulating chondrocyte functions. The observation that this HA-based preparation appears to better perform in patients with mild-to-moderate disease, where some tissue response and possibly repair can still be stimulated, further supports the just briefly outlined pharmacological profile. Not surprisingly, therefore, although the most common regulatory status for HA preparations is that of 'medical device', in Europe this HA-based tool was classified as a 'medicinal product'.

Can these observations be extended to whatever else HA-based preparation? Comparative studies suggest a negative answer^{141,176,185}. The available HA-based tools for intra-articular therapy seem to exhibit differences and specificities in many aspects of potential clinical relevance. In this respect, the analysis of the results obtained with 500-730 kDa HA suggests that factors likely deserving consideration to characterize a HA-based tool include the main mechanism of action, kinetics features and interaction with joint tissues, the subpopulation of patients expected to obtain the highest benefit from the treatment, safety issues and impact on disease-cost. Thus, current findings suggest that explanation of real-world clinical outcomes for intra-articular HA may go beyond the simple inclusion in a single, homogeneous, therapeutic class and require a more stratified categorization. Unfortunately, a great many of the currently available HA-based

preparations are endowed with a limited or absent scientific documentation aimed at characterizing their specific features^{43,44}. Therefore, additional research is needed to better document and to find out more about which aspects of intrinsic HA product properties and external factors may influence clinical outcomes associated with intra-articular HA in the real-world clinical practice.

Conflict of Interest

The Author declares that there are no conflicts of interest.

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