

Anti-inflammatory and Structure-protective Effects of Hyaluronans:

Are These Effects molecular weight dependent?

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Running title: Role of MW in different effects of HA

Abstract

Although hyaluronans (HA) has been proved to be effective in the treatment of patients with osteoarthritis and rheumatoid arthritis, the correlations between these effects and the molecular weight (MW) of HA have not been systematically followed. Many different HA preparations are now applied worldwide in clinical usage. Their molecular weights are very wide ranged (500-6000 kDa). No systematic review especially addresses on the role of HA's MW in the effects of anti-inflammation and structure-protection. This study evaluates the literatures of the basic and clinical studies on biological, pathological, and clinical effects of different MW HA. Databases were searched through PubMed (period 1978-2009), using the terms hyaluronan, hyaluronic acid, arthritis, and molecular weight. Reference lists of relevant articles were controlled for additional references. We define the "high" MW as MW greater than 2000 kDa and define the "low" MW as MW less than 2000 kDa in the current study. Most data that support the structure-protective effect of HA are from the studies with low MW HA. On the other hand, the majority of data that recommend the anti-inflammatory effect of HA are from the reports of high MW HA. In conclusion, we suggested that the effects of LMW HA were more structure-protective and those of HMW HA were more anti-inflammatory.

Keyword: Hyaluronans, Inflammatory, Molecular weight, Structure-protective

1. Introduction

Hyaluronic Acid (HA), or Hyaluronan, a linear glycosaminoglycan consisting of alternating disaccharide unit structure of D-glucuronic acid and N-acetyl-glucosamine, is a main component of synovial joint fluids and the extracellular matrix of articular cartilage.^{1,2} (Figure 1) On the level of molecular biology, HA is manufactured by a group of integral membrane proteins called hyaluronan synthases (HAS). Three human HA synthase (*HAS 1*, *HAS 2* and *HAS3*) genes have been cloned.³ HA with higher molecular weight (2×10^6 Da) is synthesized by *HAS 1* and *HAS 2*, whereas HA with lower molecular weight ($2-3 \times 10^5$ Da) is synthesized by *HAS 3*.⁴ Regarding the degradation of HA, a class of enzymes named hyaluronidases (HYAL) are involved. Up to now, six associated genes, including *HYAL1*, *HYAL2*, *HYAL 3*, *HYAL 4*, *PH-20/SPAM1*, and *HYALPI* are cloned in human.⁵

Nonsurgical treatments for osteoarthritis (OA) may be characterized as symptom-modifying or disease-modifying drugs. As defined by the Osteoarthritis Research Society (OARS), disease-modifying drugs are those that are intended to prevent, retard, stabilize, or reverse development of the morphological changes of OA.⁶ Hyaluronan has been proven to be a safe and effective symptom-modifying treatment for OA of the knee, in terms of significantly reducing pain and improving function.⁷⁻¹⁰ The original concept for viscosupplementation with HA was based on the

hypothesis that HA could help fluid replacement and restore the visco-elasticity of the synovial fluid.¹¹ However, the fact that all injected HAs are gone within days¹² and yet the clinical benefit lasts for months suggests that there may be the other mechanism through which HAs mediate their clinical benefit. It is now believed that biologic activation of multiple protective mechanisms may explain the long-term clinical benefits¹³⁻¹⁵. Substantial evidence further recommended that HA in certain patient populations can also have disease-modifying activity.¹⁶ To date, there is no systematic review especially addresses on the role of HA's MW in its effects of symptomatic and structural modifications

Some previous studies reported that, in arthritic joint, the average molecular weight and concentration of HA in the synovial fluid is reduced.^{17,18} Other scholars¹⁹ further recommended that the high MW HA seems to be depolymerized and degraded into low MW HA in the course of disease process, implying joints with higher MW HA get better function. However, the *HAS 3* gene that synthesizes the low MW HA (200-300 kDa) had later been cloned out.^{3,4} Their findings indicated low MW HA can be created by human and is not just the degraded product of high MW HA. If low MW HA is nothing but a depolymerized product, the *HAS 3* gene that synthesizes LMW HA should be deleted during the human evolution process. Besides, the surface of articular cartilage is covered with dense horizontal type-two collagen fiber. HA

with high MW and high concentration is very sticky; theoretically, it cannot penetrate this layer and further exert some chondro-protective reactions. (Figure 2A) On the other hand, low MW HA can get better potential in penetrating into the cartilage, especially in the arthritic cartilage. (Figure 2B) Thus, we hypothesize that the phenomenon of the lower HA's MW and concentration in arthritic joint is the self-protective and self-remodeling process of human body, not just from the degraded product of high MW HA by disease progression. Furthermore, we also hypothesize that HA with differencing MW may have different contributions in the maintenance of normal joint physiology.

Intra-articular injection of HA is now applied worldwide for the treatment of arthritis. Currently, there are several different preparations of HA with very wide ranged MW (500-6000 kDa), which are marketed in the world. (Table 1) That is, there is still no consensus on the best MW of HA regarding the joints with different arthritic conditions. The differences in efficacy related to the MW of HA remain a subject of debate. In this study, we systemically review the literatures concerning the basic and clinical studies on biological, pathological, and clinical effects of different MW HA. We hopefully clarify the role of HA's MW in its effects of anti-inflammation and structure-protection.

2. Materials and Methods

2.1 Literatures Search

A review of the literature was carried out based on a PubMed search between 1978 and 2009, using the terms hyaluronan, hyaluronic acid, arthritis, and molecular weight.

2.2 Definition of High and Low Molecular Weight of HA

Before comparison, it is very important to clarify the definition of “high” and “low” MW of HA due to the purpose of this study. By reviewing the literatures, studies regarding the issue of MW of HA all did not identify the definition of High MW or Low MW. Therefore, the same HA product may be regarded as High MW in one report and as Low MW in the other. For example, in the study of Neustadt²⁰, Orthovisc[®] (MW up to 2.9×10^6 Da) was regarded as HMW; however, it was considered as LMW in the other report by Karatosun.²¹

Previous investigators⁴ have reported that HA with a higher MW (2×10^6 Da) is synthesized by HAS1 and HAS2, whereas HA with a lower MW ($2-3 \times 10^5$ Da) is synthesized by HAS3. Another report indicated that the MW in normal human synovial joint fluid could be as high as $6.3-7.6 \times 10^6$ Da.¹⁸ Because the highest MW of the current commercially available HA is only up to 6.0×10^6 Da, we define the

“high” MW as MW greater than 2×10^6 Da and define the “low” MW as MW less than 2×10^6 Da in the current study.

3. Results

3.1 LOW MOLECULAR WEIGHT HYALURONAN (MW 500-2000 kDa)

By reviewing the literatures, most studies providing the evidence of structural modification by HA were conducted with low MW HA. Listrat²² conducted a prospective, controlled study of one-year duration to evaluate the potential structure-modifying effects of low MW HA (Hyalgan[®] MW 500-730 kDa). After randomization, either conventional therapy or three cycles (every 3 months) of three intra-articular injections of Hyalgan[®] (once a week during 2 weeks) were given. The structural outcome was estimated by using the x-rays: joint space narrowing and the arthroscopic findings. Thirty-six patients completed the one-year trial (19 in the Hyalgan[®] group and 17 in the control group). They found that deterioration in the structural parameters was less in the Hyalgan[®] group, suggested that repeated intra-articular injections of Hyalgan might delay the structural progression of the disease. Takahashi²³ evaluated the effect of intraarticular injections of low MW HA (Artz[®]: MW 620-1170 kDa) on chondrocyte apoptosis and NO production using an experimental OA animal model. Their findings showed that Low MW HA protects against chondrocyte apoptosis during the development of OA, while it may not have

definite effects on NO production in the joints. They recommended that these inhibitory effects of low MW HA on chondrocyte apoptosis may play a role in its mechanism of action in chondroprotection. However, the anti-inflammatory effect of low MW HA is not so dominant, in terms of no significant reduction of NO production in OA joint. Guidolin²⁴ carried out a histomorphometric study on cartilage samples taken from osteoarthritic human knees before and six months after intraarticular injections of low MW HA (MW: 500–730 kDa). The results obtained with low MW HA were compared with the results of methylprednisolone acetate treatment. Twenty-four subjects with primary OA of the knee were considered. They found that a significant reconstitution of the superficial layer was observed together with an improvement in chondrocyte density and territorial matrix appearance after six-month low MW HA treatment. In addition, chondrocytes appeared significantly improved in their metabolism, as indicated by the increased extension of the synthetic structures and mitochondria with respect to the organelles having catabolic or storage functions. Their results suggested that low MW HA treatment possessed superior chondro-protective potential to methylprednisolone. Amiel²⁵ analyzed the long-term effect of low MW HA (Hyalgan® MW: 500–730 kDa) on osteoarthritis progression using the rabbit anterior cruciate ligament transection (ACLT) OA model. They compared the gross morphological and histomorphometric changes between groups

treated with one or two courses of five weekly intra-articular injections of low MW HA, or vehicle. The gross morphological and histomorphometric evaluations were performed on harvested knee joints following sacrifice at 26 weeks after ACLT surgery. They discovered that rabbits receiving one or two courses of low MW HA injections showed less disease progression than rabbits treated with ACLT alone or with 10 vehicle injections. They concluded that low MW HA injections reduced the degree of articular degeneration in a rabbit ACLT model of OA. Ding²⁶ carried out an animal study to examine the effects of low MW HA (MW 1500 kDa) on three-dimensional microarchitecture of subchondral bone tissues in guinea pig primary OA. Their results indicated that low MW HA effectively protects against cartilage degeneration, decreases subchondral bone density and thickness, changes trabecular structure toward rod-like, so that subchondral bone becomes more compliant and thereby reduces cartilage stress during impact loading. Furthermore, low MW HA preserved cancellous bone mechanical properties by increasing bone mineralization. They recommended that early low MW HA administration is effective for intervention of OA initiation and progression, and short-term early low MW HA treatment is sufficient to maintain treatment effects. The detailed summarized data were shown as Table 2.

3.2 HIGH MOLECULAR WEIGHT HYALURONAN (MW 2000-6000 kDa)

A recent study by Yasuda²⁷ recommended that HMW HA (2700 kDa) could down-regulate the catabolic action of fibronectin fragments in rheumatoid arthritis (RA) joints as a potent inhibitor of nuclear factor (NF)- κ B which plays a key role in cytokine-mediated induction of inflammation.²⁸ Recently, Campo²⁹ also investigate the anti-inflammatory effect of HA with different molecular mass in mouse OA experiment model. They found that HMW HA (5000kDa) can provide significant anti-inflammatory effect, whereas LMW HA (50 kDa and 1000 kDa) cannot. Besides, some investigators^{30, 31} suggest that HMW HA has a greater pain-relieving effect when compared with LMW HA. For instance, Wobig³⁰ conducted a 12-week, double-masked, randomized, multicenter study to compare the effects between HMW HA (6000kDa) and LMW HA (750 kDa) treatment. Their results demonstrated that HMW HA had a significantly greater pain-relieving effect than did the LMW HA. In addition, Gotoh³¹ studied the effects of the molecular weight of HA and its action mechanisms on experimental joint pain in rats model. They suggested that HA with MW less than 40 kDa cannot produce an analgesic effect. The detailed summarized data regarding the evidences of anti-inflammatory and pain-relieving effects by high MW HA were shown in Table 3.

4. Discussion

4.1 THE PAIN-RELIEVING MECHANISM OF HA

We consider the pain-relieving effect of HA is through the mechanism of anti-inflammation over synovium, but not directly through the pain related receptors, which is supported by the following rationales and evidences: First, articular cartilage is both avascular and aneural. Consequently, the pain of OA must come from a different source. The pain generation in OA joints could be ascribed to the following causes: Tendonitis over surrounding soft tissue envelope and muscle strain caused by deformity of OA joint will induce pain; Irritation of subchondral nerve endings resulting from cartilage defect or chondromalacia will induce pain; Mechanical irritation rising from loose body or meniscus tear will induce pain.³² However, all of these factors will not be corrected in a short period by nonsurgical method; therefore, the mechanism of rapid symptom relief after HA injection should be associated with the other factor, i.e. the synovitis. (Figure 3) The inflammation of synovium could subside in few days after nonsurgical treatment. In an animal study by Qiu et al,³³ the messenger ribonucleic acid (mRNA) expression of matrix metalloproteinase (MMP)-3 in synovium was significantly suppressed after HA injection. However, in cartilage, the MMP-3 level did not significantly change. Their results also suggest that the rapid therapeutic effect of HA may be through the synovium, but not directly through the cartilage. Second, Gotoh³⁰ studied the action mechanisms of HA and recommended that HA did not interfere with the analgesic action of the bradykinin antagonist,

indicating that HA does not directly bind with bradykinin receptors. They concluded these effects of HA appear to be caused by the interaction between HA and HA receptors.

4.2 Effect of HA May Be Due To an Intrinsic Carbohydrate-Mediated Mechanism

Recently, Castro ³⁴ conducted an animal study to study the analgesic activity of a polysaccharide in experimental osteoarthritis in rats. They compared the analgesic effects of intra-articular injected galactomannan polysaccharide derived from Guar gum (GG) and high molecular weight HA (Hylan G-F20). They found that GG, either as a gel or solution, significantly inhibited joint pain similar to the inhibition achieved with Hylan G-F20. This analgesia is independent of the colloidal state. They recommend that the analgesic benefit of viscosupplementation, such as HA injection, may be due to an intrinsic carbohydrate-mediated mechanism rather than to the rheologic properties of the material. We completely agree with their recommendation. Actually, a lot of cell surface receptors and signal transduction molecular are belonging to the glucosaminoglycans (GAGs) family. The HA is also a kind of GAGs. We considered that the effect of HA is also through the intrinsic carbohydrate-mediated mechanism as well. HA may occupy the sites of cell surface receptors and then block or change the cell behaviors. In other words, after HA occupy most of the receptor engaging sites on cell surface; other molecular cannot

interact with the cell due to limited surface receptor left. Therefore, HA may stabilize the cell through this mechanism.

5. Conclusion

Currently, no pharmacological managements for OA are approved for the indication of modifying the rate of OA progression. However, evaluation of novel agents and agents with established symptom-relieving activity for structure-modifying effects has become a main focus of research in arthritis. By reviewing the literatures, we recommended that the effects of LMW HA were more structure-protective and those of HMW HA were more anti-inflammatory.

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Table 1. Characteristics of marketed hyaluronans

Product	Derivation	Content	MW(kDa)	N.I.	Q.P.I (ml)
Hyalgan®	Rooster combs	20 mg sodium hyaluronate	500–730	3 or 5	1
Artzdispo®	Rooster combs	25 mg sodium hyaluronate	600-800	5	2.5
Supartz®	Rooster combs	25 mg sodium hyaluronate	620–1,170	5	2.5
Lumisteron Dispo®	Rooster combs	25 mg sodium hyaluronate	600-1,200	5	2.5
Ostenil®	Bacterial fermentation	20 mg sodium hyaluronate	1,000-2,000	3	2
Orthovisc®	Rooster combs	30 mg sodium hyaluronate	1,000-2,900	3 or 4	2
EuXexxa®	Bacterial fermentation	20 mg sodium hyaluronate	2,600-3,400	3	2
Synvisc®	Rooster combs (chemically modified or cross-linked)	16 mg sodium hyaluronate derivative	80%: 6,000; 20%: >6,000	3	2

MW: Molecular weight

Q.P.I : Quantity per injection, N.I.: Number of injection

Table 2 Evidences of chondro-protective effect by LMW HA

Authors	Year	Design	HA product (MW)	Outcome factors	Results
Listrat et al. ²²	1997	Clinical study	Hyalgan [®] (500-730 kDa)	Joint space/Arthroscope and radiograph	LMW HA treated group showed less deterioration of joint space
Takahashi et al. ²³	2000	Animal study (Rabbit)	Artz [®] (620-1170 kDa)	Chondrocytes / apoptotic cell death	LMW HA against chondrocyte apoptosis
Guidolin et al. ²⁴	2001	Clinical study	Hyalgan [®] (500-730 kDa)	Cartilage biopsy/ Electron microscope	LMW HA reconstitutes superficial layer of cartilage , improves chondrocyte density and metabolism
Amiel et al. ²⁵	2003	Animal study (Rabbit)	Hyalgan [®] (500-730 kDa)	Gross morphology and histology of cartilage	LMW HA reduced the degree of articular degeneration; repeat injections were best
Ding et al. ²⁶	2005	Animal study (guinea pig)	Lifecore Biomedical, Inc.(1500 kDa)	3-D microarchitecture of subchondral bone plate, cancellous bone and cortical bone; mechanical testing and collagen and mineral determinations	LMW HA protected against cartilage degeneration, preserved cancellous bone mechanical properties by increasing bone mineralization.

Table 3 Evidences of anti-inflammatory and pain-relieving effects by HMW HA

Authors	Year	Design	HA product (MW)	Outcome factors	Results
Gotoh et al. ³⁰	1993	Animal study (Rats)	HA (6.8, 40,310, 860,2300 kDa)	Behavior grading criteria for evaluating pain reaction	HMW HA produced high and long-lasting analgesia than LMW HA.
Wobig et al. ²⁹	1999	Clinical study	Hylan [®] G-F 20 (6000 kDa) vs LMW HA (750 kDa)	Weight-bearing pain, most painful knee movement, and overall treatment response (using a visual analogue scale)	HMW HA G-F 20 had significantly greater pain-relieving effects than did the LMW HA
Yasuda et al. ²⁷	2007	Basic study (Human)	HMW HA (2700 kDa)	Inhibitory effect of HMW HA on nuclear factor (NF)-kappaB activation	HMW HA significantly suppressed heparin-binding fibronectin fragment -activated NF-kappaB
Campo et al. ³¹	2009	Animal study (mouse)	LMW HA (50 kDa); medium MW HA (1000 kDa); HMW HA (5000 kDa)	Levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, interferon (IFN)- γ and iNOS gene expression	Only HMW HA can reduce all the detrimental effects stimulated by lipopolysaccharide treatment

Legends:

Figure 1: The repeating molecular unit of hyaluronic acid (HA)

Figure 2A: The normal articular cartilage is covered with dense horizontal type-two collagen fiber. Theoretically, HA cannot penetrate this layer and exert some further reactions.

Figure 2B: The surface of arthritic cartilage was damaged and the dense horizontal type-two collagen fiber was torn. There are some fissures through the surface layer and being into the deep layer of the cartilage. HA with low molecular weight can get better potential in penetrating into the cartilage.

Figure 3: Under arthroscope, the synovitis was apparent in arthritic joint.