

A Review Paper

Hyaluronans: Is Clinical Effectiveness Dependent on Molecular Weight?

Peter C. Vitanzo, Jr, MD, and Brian J. Sennett, MD

Abstract

The original rationale for viscosupplementation with hyaluronans was fluid replacement, suggesting that the most viscous materials (eg, those of highest molecular weight [MW]) would provide the most clinical benefits. However, it has become clear that mechanisms of action for osteoarthritis pain management are not only mechanical but also biological. After intra-articular injection, hyaluronans exert a range of biological actions within the joint. Although high- and low- to mid-MW hyaluronans (but not hyaluronans <500 kDa MW) are more or less active (depending on the specific effect examined), it is not known which actions are clinically meaningful. There is no evidence for a difference between hyaluronan products in clinical efficacy measured as pain relief, but investigators in several preclinical studies evaluating joint-structure modification in osteoarthritis models have reported advantages to using low- to mid-MW hyaluronans.

Hyaluronan intra-articular injections are effective and well tolerated for the treatment of osteoarthritis of the knee in patients who either do not respond adequately to conventional therapy or who are intolerant of nonsteroidal anti-inflammatory drugs.¹ Patients with osteoarthritis tend to have a reduced concentration and lower mean molecular weight (MW) of hyaluronan in their synovial fluid, which led to the idea that joint fluid replacement (viscosupplementation) with exogenous hyaluronan would be beneficial.² This hypothesis predates discovery of both endogenous hyaladherins (now known to be responsible for natural cross-linking of hyaluronans in synovial fluid and extracellular cartilage matrix) and hyaluronan cell-surface receptors.³

It has been reported that elastoviscosity and joint retention of exogenous hyaluronan increase with MW.⁴ This finding led to the proposal that duration of clinical benefit was proportional to MW on the basis of increased viscosi-

ty (mechanical and lubrication function), duration of residence in the joint, and stimulation of endogenous hyaluronan by high-MW hyaluronans. However, results of preclinical studies, in which hyaluronans of differing MWs have been compared, suggest that beneficial effects on pathogenesis and progression of osteoarthritis are seen across the range of MWs of available compounds (Table I),⁵⁻¹⁷ with some researchers finding higher levels of apparent efficacy with low- to mid-MW compounds.

"Regulation of hyaluronan expression and function is complex."

CD44, a membrane-bound glycoprotein, is one of several identified hyaluronan-binding receptors seemingly involved in regulating hyaluronan synthesis and other biological activities associated with hyaluronan. Regulation of hyaluronan expression and function is complex. Three related genes of the hyaluronan synthase gene family are differentially regulated and synthesize hyaluronans of mean MW of 200 kDa or 2000 kDa. Nascent hyaluronans are bound together in the extracellular fluid by a family of hyaluronan-binding proteins termed hyaladherins,³ increasing their MW and resultant viscosity. Each hyaluronan molecule may have as many as 200 hyaladherin molecules associated with each chain.

Results from preclinical studies of potential joint-structure modification in animal models of osteoarthritis have shown that, compared with high-MW hyaluronans, low- to mid-MW hyaluronans (500–2000 kDa) may have an advantage in that they can access diseased tissues more readily.^{11,18-20} Coleman and colleagues¹⁹ showed that, when hyaluronans of various MWs were infused into the knees of anesthetized rabbits, the flow-buffering effect of hyaluronan depended on molecular chain length, with chain length shortening associated with reduced molecular reflection by the synovial interstitium. With the 2 shortest chains (90 kDa, 300 kDa), fluid drainage was reduced relative to Ringer's solution but increased steeply with pressure, whereas the fluid drainage rate became relatively insensitive to pressure when chain length was increased (to 530 kDa and 2200 kDa).

Prospective, controlled, and comparative studies have yielded no consistent evidence supporting the premise that increases in MW or viscosity are associated with increases

Dr. Vitanzo is Specialist, Nonoperative Sports Medicine, Rothman Institute, Thomas Jefferson University, Philadelphia, Pennsylvania.

Dr. Sennett is Assistant Professor, Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, Pennsylvania.

Address correspondence to Brian J. Sennett, MD, Penn Sports Medicine, Weightmann Hall 235, University of Pennsylvania Health System, S 33rd St, Philadelphia, PA 19104 (tel, 215-615-4400; fax, 215-615-4405; e-mail: brian.sennett@uphs.upenn.edu).

Hyaluronans: Is Clinical Effectiveness Dependent on Molecular Weight?

in clinical efficacy, as shown by outcomes such as pain relief. Data from recent clinical studies carried out in patients receiving a low- to mid-MW product have suggested that intra-articular hyaluronan therapy has disease-modifying activity and may alter progression of osteoarthritis.²¹⁻²⁵ These issues are discussed in more detail later in this review.

PROPOSED MECHANISMS OF ACTION OF HYALURONAN

Hyaluronans are responsible for elastoviscosity of synovial fluid in joints, and elastoviscosity is higher with high-MW hyaluronans. This elastoviscosity is shear-dependent, meaning that the mechanical behavior of hyaluronan changes with the shear force applied and the speed of flow: Hyaluronan solutions behave as viscous fluids when external forces move them at low speeds but behave as elastic bodies when subjected to high rates of shear or frequency. Therefore, hyaluronans are effective as lubricants under conditions of slow movement but are effective as shock absorbers when movement is rapid.⁴

Clearly, this elastoviscous behavior will determine clinical activity to a large extent, but the pharmacokinetic profile of hyaluronans does not support a purely mechanical role for these substances. During movement, hyaluronan flows into the lymphatic system of the joint capsule, moves into the general circulation, and is ultimately taken up by the liver, where it is degraded to water and carbon dioxide.²⁶ Duration of the therapeutic effect of hyaluronan, as noted in clinical studies, is also not consistent with a purely mechanical role, as treatment benefits can persist for several months after a course of injections is completed, whereas the elimination half-life of hyaluronan in the joint is measured in hours to days.^{27,28}

"...the pharmacokinetic profile of hyaluronans does not support a purely mechanical role for these substances."

Of additional interest is the reported increase in the elastoviscous properties of synovial fluid after exogenous administration of hyaluronic acid. Mensitieri and Ambrosio²⁹ examined synovial fluid from the knee joints of patients subjected to arthrocentesis or treatment with hyaluronan compounds of MWs ranging from 500 kDa to 730 kDa. The elastoviscosity of synovial fluid from patients who received hyaluronan was increased relative to pretreatment values, as well as to values in patients who had undergone arthrocentesis. This increase in elastoviscosity was present 1 week after administration, well after the exogenous product would have been expected to have been cleared from the joint space, suggesting that the elastoviscosity of knee synovial fluid benefits from increases

in both hyaluronic acid concentration and MW.

Other potential pharmacologic mechanisms may be receptor-mediated. These mechanisms include inhibition of inflammatory mediators, inhibition of phagocytic cell function, stimulation of cartilage matrix synthesis, and decreased degradation of cartilage matrix.^{17,30-39}

These and other potential mechanisms, and their association with MW, investigated primarily in vitro, are summarized in Table II.^{17,30-39} Results from in vitro evaluation of the biological actions of hyaluronans show that both high- and low- to mid-MW hyaluronans are more or less active depending on the specific effect examined. For example, mid-MW hyaluronans (500–999 kDa) appear to inhibit apoptosis more,³⁵ offer more protection against cartilage loss,¹⁷ and suppress synovial cell proliferation more,^{11,17} whereas high-MW hyaluronans are more effective in inhibiting prostaglandin E2 and arachidonic acid activities.^{37,38} Both agents are similar with regard to effects on leukocyte chemotaxis.³⁰ Although an "ideal" MW range should be related to the biological profile that will optimize clinical benefit for the patient, it is not well understood which of these actions are most meaningful regarding clinical benefits, such as pain relief or potential structure (disease) modification. Potential disease-modifying activities demonstrated in animal models of osteoarthritis (Table I) may provide a more meaningful picture of the benefit of low- to mid-MW hyaluronans. Even in this case, however, the activity may be distinct from the outcome of pain relief. In a review, Ghosh and Guidolin¹⁸ concluded that, based on overall preclinical and clinical data, low- to mid-MW hyaluronans used clinically for osteoarthritis may indeed have a more beneficial structure- or disease-modifying profile.

RELATIONSHIPS BETWEEN MOLECULAR WEIGHT OF HYALURONAN AND EFFICACY

In the United States, hyaluronan products marketed for treatment of osteoarthritis of the knee include Hyalgan (sodium hyaluronate; sanofi-aventis, Bridgewater, NJ), Synvisc (hylan G-F 20; Genzyme Corporation, Cambridge, Mass), Supartz (sodium hyaluronate; Smith & Nephew, Memphis, Tenn), Euflexxa (sodium hyaluronate; Ferring Pharmaceuticals, Suffern, NY), and Orthovisc (high-MW hyaluronan; DePuy Mitek, Raynham, Mass). Characteristics of these preparations are summarized in Table III.

Molecular Weight and Half-Life

Exogenous hyaluronan begins to leave the joint within 2 hours of injection, though some remains up to 3 days (this is particularly true of high-MW products). Synvisc remains up to 3 days,⁴⁰ whereas Hyalgan,⁴¹ Supartz,⁴² Euflexxa,⁴³ and Orthovisc⁴⁴ remain less than 24 hours. Although the high-MW hylan B in Synvisc has a half-life of some weeks,²⁶ it is biologically inert (not in solution to engage receptors). Results from a study involving ¹³¹I-radiolabeling showed that hyaluronan is eliminated from the human

Table I. Preclinical Studies of Intra-Articular Hyaluronan (HA) Preparations in Animal Models of Osteoarthritis (OA)*

Study	Species	OA Induction Method and Subsequent Therapy	HA Origin (MW)	Findings
Asari et al ¹¹	Beagle	ACLT, then HA × 5 wk (starting 4 wk PO)	Seikagaku (840 kDa or 2300 kDa)	Pathology ↓ significantly more with 840 kDa than with 2300 kDa. Synovial penetration of 840 kDa > 2300 kDa
Ghosh et al ¹²	Sheep	UTMM, then HA or saline × 5 wk (starting 4 mo PO)	Seikagaku (840 kDa); Nippon Roussel (2000 kDa)	Loading of operated joints ↑ and radiologic scores ↓ with HAs vs saline. Histologic scores for MTPC worse with 2000 kDa
Ghosh et al ¹³	Sheep	UTMM, then HA or saline × 5 wk (starting 4 mo PO)	Seikagaku (840 kDa); Nippon Roussel (2000 kDa)	PG synthesis in MTPC of operated joints ↓ with HA vs placebo, but PG release ↑
Adam ¹⁴	Sheep	TBLM, then HA or saline × 5 wk (starting 4 mo PO and after SF aspiration)	Seikagaku (840 kDa or 2300 kDa)	SF HA MW (determined by multiangle laser light-scattering photometry) ↑ at 5 wk PO with both HAs, but mean ↑ with 840 kDa > 2300 kDa
Ghosh et al ¹⁵	Female sheep	TBLM, then HA or saline × 5 wk (starting 4 mo PO and after SF aspiration)	Fidia (500–730 kDa); Pharmacia-Upjohn (3000–6900 kDa)	SF storage modulus and viscosity at 5 wk PO ↑ vs saline with both HAs. Improvement with 500–730 kDa > 3000–6900 kDa
Yoshimi et al ¹⁶	Rabbit	ACLT, then single-dose HA or saline	Hikari (2020 kDa or 950 kDa)	Both HAs protected against histologic cartilage loss. Nonsignificant trend toward greater efficacy with 2020 kDa
Kikuchi et al ⁷	Rabbit	PM, then HA or saline immediately PO 2x/wk × 2–4 wk	Seikagaku (840 kDa or 1900 kDa)	Cartilage histology suggested greater protection with 840 kDa than with 1900 kDa or saline
Shimizu et al ¹⁷	Rabbit	ACLT, then HA × 5 wk (starting 4 wk PO)	Fidia (500 kDa); Seikagaku (840 kDa); Pharmacia (3600 kDa); Biomatrix (6000 kDa)	Protective effect in cartilage and synovium: higher MW > MW 500–730 kDa. Suppression of synovial cell proliferation: 840 kDa > larger HAs.

*Investigations shown are those in which HAs with differing MWs were compared directly. Compounds were administered intra-articularly in all studies. MW indicates molecular weight; ACLT, anterior cruciate ligament transection; PO, postoperatively; UTMM, unilateral total medial meniscectomy; MTPC, medial tibial plateau cartilages; PG, proteoglycan; TBLM, total bilateral lateral meniscectomy; SF, synovial fluid; PM, partial meniscectomy.

knee joint in 3 distinct phases (half-lives of 1.5 hours, 1.5 days, and 4 weeks) fitting a 3-exponent function.⁴⁵ In this study, conducted with a high-MW-nonanimal stabilized hyaluronic acid product (NASHA), it was suggested that the rapid initial phase was linked to elimination of low-MW fragments and the second phase to elimination of high-MW labeled hyaluronan. During the slow third phase, the daily decline in radioactivity was comparable to that in the urine, indicating a slow release of hyaluronan or degradation products from the gel in the knee and subsequent excretion by the kidneys. The possibility of uptake of NASHA and its products by the synovial layer and

popliteal lymph nodes was also postulated.⁴⁵ Results from another study showed increased clearance of hyaluronan with osteoarthritis but no increase in normal joints.⁴⁶ The elimination half-life of hyaluronan increased to 23.5 hours as osteoarthritis developed but fell to 17.4 hours by the time the disease was fully established.

Molecular Weight and Tissue Penetration

The discrepancy between retention in the joint and duration of clinical effect may be attributable, at least in part, to enhanced penetration of low- to mid-MW hyaluronans into the extracellular matrices of the synovial tissues and per-

Table II. Molecular Weight and Effect* on Biological Activity of Hyaluronan

Biological Activity	Molecular Weight Range (kDa)		
	Low (<500)	Mid (500-999)	High (1000-3999)
In vitro data			
Inhibition of leukocyte chemotaxis ³⁰	-/+	+/++	++
Inhibition of phagocytosis ³⁰	-/+	++	+++
Inhibition of lymphocyte proliferation ³¹	-	+++	NT
Oxygen-derived free radical scavenging ³²	+	++	+++
Inhibition of enzymatic cartilage degradation ^{33,34}	-	-/+	+/++
Inhibition of apoptosis ³⁵	-	++	+
Stimulation of endogenous hyaluronan production ³⁶	-	+++ [†]	+++ [†]
Inhibition of prostaglandin E2 and arachidonic acid activities ^{37,38}	+	+	+++
In vivo animal studies			
Analgesia ³⁹	-/+	NT	+++
Protection against cartilage loss ^{†† 7,17}	NT	+/+/+++	+/+++
Suppression of synovial cell proliferation ^{‡11,17}	NT	+/+++	+

*Minus sign (-) indicates no significant effect; plus sign (+), mild effect; ++, moderate effect; +++, marked effect; NT, not tested.

[†]Smith and Ghosh³⁶ found that maximal stimulation of hyaluronan synthesis of human synovial cells was achieved using preparations of MW 500 kDa to approximately 4000 kDa; preparations of MW less than 500 kDa had no effect; with preparations of MW 4700 kDa or more, stimulation declined with increasing HA concentration.

^{††}The 2 studies showed marked differences. Protection against cartilage loss was only mild with the 500-kDa hyaluronate or the 3600-kDa hyaluronate but was marked with the 800-kDa hyaluronate in the study by Shimizu and colleagues.¹⁷

[‡]In the study by Asari and colleagues,¹¹ protection against cartilage loss was moderate with the 800-kDa hyaluronate but was marked with the 1900-kDa hyaluronate.

Table III. Details of Hyaluronans Commercially Available in the United States

	Compound				
	Hyalgan ^{®41}	Supartz ^{®42}	Synvisc ^{®40}	Orthovisc ^{®44}	Euflexxa ^{™43}
Generic name	Sodium hyaluronate	Sodium hyaluronate	Hylan G-F 20	High-molecular-weight hyaluronan	Sodium hyaluronate
Manufacturer	sanofi-aventis	Smith & Nephew	Genzyme Corporation	DePuy Mitek	Ferring Pharmaceutical
Molecular weight (kDa)	500-730	620-1170 20% hylan B (>6000)	80% hylan A (6000),	1000-2900	2400-3600

happens the cartilage. There is some evidence—from a canine osteoarthritis model evaluating increased expression of prostaglandin E2, thickening of synovial lining layers, and vacuolar changes in lining cells, together with fluorescein staining of hyaluronan—that low- to mid-MW hyaluronans (ie, 840 kDa) penetrate diseased tissues more effectively than high-MW hyaluronans (ie, 2300 kDa). Pathologic changes suggested more accessibility of synovial tissues to the 840-kDa product.¹¹ However, there remains no direct evidence that this phenomenon correlates with or explains a clinical benefit.

Molecular Weight and Elastoviscosity

Increasing the mean MW of hyaluronan clearly increases the elastoviscous properties of the solution.⁴ Although this result would be advantageous if the mechanism of pain relief were only mechanical, it seems counterintuitive when biological mechanisms are involved. It does not follow that cellular activation of the precisely regulated hyaluronans and other associated pathways would occur in response to a higher concentration and higher than normal MW of hyaluronan in the knee. Within the cartilage matrix, structural properties of the extracellular matrix are deter-

mined by aggregates of endogenous hyaluronan and proteoglycans that include aggrecan and link proteins.⁴⁷ Hyaluronan MW seems to be determined by the binding of elongating nascent hyaluronan to a receptor. Saturation of these receptors may stall elongation of the nascent hyaluro-

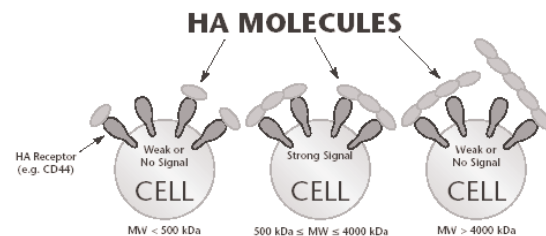


Figure. In vitro synthesis of hyaluronan by synovial fibroblasts is influenced by concentration and molecular weight (MW) of exogenous hyaluronan (HA). The signal for synthesis is provided by HA in the MW range of 500 to 4000 kDa in a concentration-dependent manner. Figure adapted with permission from Smith MM, Ghosh P. The synthesis of hyaluronic acid by human synovial fibroblasts is influenced by the nature of the hyaluronate in the extracellular environment. *Rheumatol Int.* 1987;7:113-122

nan chains, resulting in an intracellular response that affects both hyaluronan and proteoglycan synthesis.

Although an efficacy advantage has been claimed for products of a MW similar to that of normal, healthy synovial fluid, analysis of the clinical evidence has not shown a correlation between MW and degree of improvement or duration of efficacy.⁴⁸

Molecular Weight and Receptor Binding

It is thought that hyaluronan concentration is monitored by synovial fibroblasts and that homeostasis is maintained through specific cell-surface receptors. These receptors have also been shown *in vitro* to be activated by exogenous hyaluronan, with maximal responses being elicited by exogenous hyaluronans of certain size ranges.³⁶ In one study, investigators found the most marked response by synovial fibroblasts from an osteoarthritic joint exposed to hyaluronan of MW >500 kDa, whereas smaller molecules had little or no effect.³⁶ The investigators also found that a high-MW hyaluronan (4700 kDa) was less effective in stimulating synthesis than a compound of MW 3800 kDa. They thought this finding indicates that synovial fibroblasts do not increase synthesis of endogenous hyaluronans in the presence of functionally acceptable (ie, very high MW or high concentration) hyaluronans.

Study results have shown that hyaluronan binding at the cell surface is a complex interplay of multivalent binding events affected by the size of the multivalent hyaluronan ligand, the density of cell-surface CD44, and the CD44 activation state.⁴⁹ The most significant feature distinguishing CD44 from other hyaluronan-binding proteins is that, with CD44, binding to hyaluronan takes place at the cell surface, where multiple, closely arrayed CD44 receptor molecules interact with the highly multivalent repeating disaccharide chain of hyaluronan. The actual affinity of a single CD44-hyaluronan binding domain for hyaluronan is likely to be very low. Thus, binding of a CD44-positive cell to a soluble hyaluronan molecule must involve multiple weak receptor-ligand interactions, dependent on the relative valency of the native hyaluronan molecule. In high-MW hyaluronan polymers, each molecule interacts with more than one receptor-binding site, and this increases the likelihood that the molecule will remain bound to the receptor, thereby increasing the receptor affinity (avidity) of the molecule. Beyond a certain limit, however, very large hyaluronan molecules will be less efficient in engaging multiple receptors because of steric hindrance—hence the bimodal nature of many biological activities. This suggests that the maximal response would be produced by hyaluronans within a specific size range (neither too big nor too small). The existence of isoforms of the hyaluronan receptors may also contribute to differential binding. However, these ideas remain speculative at this time, and the exact mechanism of receptor binding is not known. Either the response may be proportional to the number of receptors bound, or it may be an “all-or-nothing” phenomenon that depends on the simultaneous binding of 2 or more recep-

tors.¹⁸ In addition, biological activation is also balanced with “bioavailability,” the ability to penetrate into diseased tissues. Again, larger hyaluronan molecules may be less efficient in penetrating into the synovium (see Figure).¹¹

CLINICAL EFFICACY

There have been several reviews^{1,50-52} and meta-analyses^{53,54} on the clinical efficacy of intra-articular hyaluronans in the treatment of pain associated with osteoarthritis—including, most recently, publication by the Cochrane Collaboration of the most comprehensive meta-analysis of this class.⁵⁵ However, there have been no reports of any well-controlled, randomized clinical studies directly comparing hyaluronans across a wide range of MWs (500–6000 kDa). Several clinical studies have compared 2 or more hyaluronans of differing MWs. Wobig and colleagues⁵⁶ reported on a study that was said to compare hylan G-F 20 (MW ≈ 6000 kDa) with sodium hyaluronate (MW ≈ 800 kDa) and find the high-MW hyaluronan superior. However, it was subsequently noted that the original study was a 4-arm trial that also included another sodium hyaluronate (MW ≈ 2300 kDa) and a control group of a degraded, nonelastoviscous hylan G-F 20 preparation.⁵⁷ In the total analysis, it was shown that none of the hyaluronans was statistically different from the control group. A retrospective clinical practice report compared hylan G-F 20 with sodium hyaluronate (MW ≈ 615 kDa) and found that, for both products, all patients reported a statistical improvement in pain after treatment.⁵⁸ The report further suggested that, compared with sodium hyaluronate, hylan G-F 20 had a superior response in many parameters. However, it was subsequently reported that significant limits in the scope and design of the study called its conclusions into question.⁵⁹

Three recent prospective studies from clinical practice also compared the clinical efficacy of hylan G-F 20 (MW ≈ 6000 kDa) with that of sodium hyaluronate (MW 500–730 kDa): Brown and colleagues⁶⁰ (N = 54) reported no evidence for a benefit of either product over the other, and Garcia⁶¹ (N = 51) and Richardson and colleagues⁶² (N = 90) reported in 2 separate studies that the short-term efficacy of hylan G-F 20 (MW ≈ 6000 kDa) and sodium hyaluronate (MW 500–730 kDa) could not be distinguished. However, all 3 reports documented a product-specific pseudoseptic reaction to hylan G-F 20—also termed severe acute inflammatory reaction (SAIR). None of these studies reported any SAIRs to the sodium hyaluronate injections. Incidence of SAIRs in these reports ranged from 5.3% to 20.7% of the patients injected. Similarly, Hamburger and colleagues⁶³ reported that in clinical practice study of 171 patients, 5 weekly injections of sodium hyaluronate (MW 500–730 kDa) had a mean duration of benefit of 447 days, whereas 3 weekly injections of hylan G-F 20 had a mean duration of 370 days ($P = .018$ in favor of sodium hyaluronate). Furthermore, incidence of SAIRs was 16.3% with hylan G-F 20 versus 0% for sodium hyaluronate ($P < .0001$).

Hyaluronans: Is Clinical Effectiveness Dependent on Molecular Weight?

Karlsson and colleagues⁶⁴ recently reported on a multicenter, randomized, saline-controlled study comparing the clinical efficacy of hylan G-F 20 (MW $\approx$ 6000 kDa) or sodium hyaluronate (MW $\approx$ 890 kDa) with that of an intra-articular saline control. Patients with knee osteoarthritis treated with injection of hyaluronan or saline showed clinical improvement during the first 26 weeks of treatment, though neither hyaluronan preparation demonstrated any significant difference from the saline control. Collectively, the limited clinical trial evidence suggests that there is no definitive difference in efficacy (symptomatic relief) between hyaluronans ranging in MW from 500 kDa to 6000 kDa, though there seem to be clear differences in safety profiles and other clinically relevant benefits (eg, disease-modifying) in several studies.

Kirshner and Marshall compared the safety and effectiveness of sodium hyaluronate (MW 2400-3600—kDa) prepared by biological fermentation with those of avian-derived hyaluronan (hylan G-F 20 (MW $\approx$ 6000 kDa). The effectiveness was not inferior and there was a significantly higher incidence of post-injection effusions in hylan G-F 20.⁶⁵

This conclusion was reiterated in the American College of Rheumatology 2000 treatment guidelines,⁶⁶ in which it was recommended that intra-articular hyaluronan therapy be considered a possible therapy for knee osteoarthritis: “To date, differences in clinical efficacy between these preparations [Hyalgan and Synvisc] as a function of molecular weight have not been demonstrated.” In agreement, the Cochrane meta-analysis concluded that no evidence would lead one to conclude that hyaluronan products differ in pain-relieving efficacy, though it was acknowledged that few well-designed, head-to-head studies have been conducted.⁵⁵

RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND TOLERABILITY

As a class, the hyaluronans have a well-documented tolerability profile, with no known systemic effects and few contraindications or drug interactions.⁶⁷ The most common side effect noted in most clinical studies has been injection-site pain. Although any intra-articular injection can elicit an inflammatory response, a clinically distinct reaction known as pseudosepsis or SAIRs has emerged after hyaluronan injections. Although it is not clear whether pseudoseptic reactions can occur with any hyaluronan, all published reports to date have been linked to hylan G-F 20 (Synvisc). Whereas the manufacturing practices for all hyaluronan products have similar quantitative specifications for the amount of allowable proteins within the products (generally $<0.1\%$), qualitatively the protein contaminants in hylan G-F 20 that have been exposed to potential cross-linking by formaldehyde and vinylsulfone have been shown to be immunologically distinct.⁶⁷⁻⁶⁹ Indeed, cross-linking is known to enhance or modify the immunogenicity of antigens, and rabbit studies have demonstrated an inflammatory reaction to hylan but not to sodium hyaluronate preparations after injection into the joint space.⁷⁰ Interestingly,

there have been several case reports of patients having a SAIR to hylan G-F 20 and subsequently being treated with sodium hyaluronate with good clinical results and without further sequelae,⁷¹ adding further support for a hylan G-F 20-specific reaction.

Antibodies to hylan have been noted in the sera of some patients.⁷¹ In addition, the immunologic response may progress to more serious conditions such as granulomatous reactions. Cases of hylan G-F 20-related granulomatous reactions have been reported in patients who had knee osteoarthritis treated with intra-articular injections of this agent.⁷¹ The reactions take the form of chronic inflammation of the perisynovial area and surrounding tissues and are shown by histologic evaluation to include histiocytic and foreign body giant cells. A recent report also characterized this response as a pseudosarcoma.⁷² A recent animal study comparing the biocompatibility of sodium hyaluronate and saline with hylan G-F 20 has confirmed these clinical observations.⁷³ After intradermal injection in guinea pigs and intramuscular injection in rabbits, hylan G-F 20 induced definitive macroscopic changes in guinea pigs by day 14 and in rabbits by day 28. Severe granulomatous inflammation in guinea pigs and acute inflammation with minimal infiltration of macrophages and foreign body giant cells in rabbits were seen on histologic assessment. Furthermore, specific antibodies against hylan G-F 20 were demonstrated in guinea pigs by passive cutaneous anaphylaxis, and substantial deposits of immunoglobulin G on hylan G-F 20 were evident by immunohistochemistry.⁷³

CONCLUSIONS

Data on intra-articular hyaluronan therapy for osteoarthritis pain—which have been accumulating for almost 2 decades—have shown that the original (1980s) hypothesis of a link between hyaluronan MW and duration of efficacy is not borne out by preclinical and clinical evidence. It is instead likely that the mechanism of action of hyaluronan is based on several probable biological activities mediated through receptor-based pharmacologic mechanisms that go beyond the physical, mechanical, and elastoviscous properties of these products.

Current evidence suggests that there is an optimal MW range for induction of various biological activities. In primarily *in vitro* analyses, some of these activities are induced more effectively by high-MW hyaluronans, whereas others are induced by low- to mid-MW hyaluronans. In contrast, preclinical studies evaluating joint-structure outcomes in animal models of osteoarthritis indicate that low- to mid-MW hyaluronans may have more potential for disease modification. There is no solid evidence for a difference between hyaluronan products in clinical efficacy as indicated by pain reduction.⁵⁵ However, additional head-to-head studies should be considered to firmly establish any possible benefits in terms of efficacy between products of differing MWs, particularly in distinct patient populations. It would be particularly important also to evaluate any differences with regard to a potential impact on osteoarthritis

disease progression, which has been described for low- to mid-MW sodium hyaluronate (500–730 kDa).^{22-25,74,75} When risk–benefit assessment is used in making treatment decisions, lack of evidence for a superior clinical benefit from use of high-MW hyaluronans versus low- to mid-MW hyaluronans should be considered together with the finding that cross-linked polymer products may be associated with increased risk of adverse events in the knee, specifically SAIRs and granulomas.

AUTHOR ACKNOWLEDGMENTS AND DISCLOSURE STATEMENT

This study was supported by a grant from Sanofi-Synthelabo. Dr. Vitanzo wishes to note that he is a consultant and lecturer for sanofi-aventis and for Smith & Nephew. Dr. Sennett wishes to note that he has received a grant from sanofi-aventis.

REFERENCES

- Altman RD. Status of hyaluronan supplementation therapy in osteoarthritis. *Curr Rheumatol Rep*. 2003;5:7-14.
- Balazs EA, Denlinger JL. Clinical uses of hyaluronan. *Ciba Found Symp*. 1989;143:265-275.
- Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med*. 1997;242:27-33.
- Balazs EA, Denlinger JL. Viscosupplementation: a new concept in the treatment of osteoarthritis. *J Rheumatol Suppl*. 1993;39:3-9.
- Shimizu C, Yoshioka M, Coutts RD, et al. Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. *Osteoarthritis Cartilage*. 1998;6:1-9.
- Yoshioka M, Shimizu C, Harwood FL, Coutts RD, Amiel D. The effects of hyaluronan during the development of osteoarthritis. *Osteoarthritis Cartilage*. 1997;5:251-260.
- Kikuchi T, Yamada H, Shimmei M. Effect of high molecular weight hyaluronan on cartilage degeneration in a rabbit model of osteoarthritis. *Osteoarthritis Cartilage*. 1996;4:99-110.
- Armstrong S, Read R, Ghosh P. The effects of intraarticular hyaluronan on cartilage and subchondral bone changes in an ovine model of early osteoarthritis. *J Rheumatol*. 1994;21:680-688.
- Schiavinato A, Lini E, Guidolin D, et al. Intraarticular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs. II. Morphological findings. *Clin Orthop*. 1989;241:286-299.
- Wenz W, Graf J, Brocai DR, et al. Wirksamkeit von intraartikular applizierter Hyaluronsäure auf Frühformen der Femoropatellararthrose—Eine experimentelle Untersuchung an Hunden. *Orthop Ihre Grenzgeb*. 1998;136:298-303.
- Asari A, Miyauchi S, Matsuzaka S, Ito T, Kominami E, Uchiyama Y. Molecular weight-dependent effects of hyaluronate on the arthritic synovium. *Arch Histol Cytol*. 1998;61:125-135.
- Ghosh P, Read R, Numata Y, Smith S, Armstrong S, Wilson D. The effects of intra-articular administration of hyaluronan in a model of early osteoarthritis in sheep. II. Cartilage composition and proteoglycan metabolism. *Semin Arthritis Rheum*. 1993;22(suppl 1):31-42.
- Ghosh P, Read R, Numata Y, Smith S, Armstrong S, Wilson D. The effects of intra-articular administration of hyaluronan in a model of early osteoarthritis in sheep. I. Gait analysis, radiological and morphological studies. *Semin Arthritis Rheum*. 1993;22(suppl 1):18-30.
- Adam N. Studies on Hyaluronan and Glycosaminoglycans of Normal and Pathological Synovial Fluid [master's thesis]. Sydney, Australia: University of Sydney; 1999.
- Ghosh P, Kipic B, Swain M, Smith MM, Cake M, Read R. Improvement in rheological parameters of synovial fluids aspirated from OA joints of sheep administered intra-articular hyaluronan (MW = 0.5-0.73 x 10⁶ Da) but not a higher MW preparation. In press.
- Yoshimi T, Kikuchi T, Obara T, Yamaguchi T, Sakakibara Y, Ito T. Effects of high-molecular-weight sodium hyaluronate on experimental osteoarthritis induced by the resection of rabbit anterior cruciate ligament. *Clin Orthop*. 1994;298:296-304.
- Shimizu C, Kubo T, Hirasawa Y, Coutts RD, Amiel D. Histomorphometric and biochemical effect of various hyaluronans on early osteoarthritis. *J Rheumatol*. 1998;25:1813-1819.
- Ghosh P, Guidolin D. Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent? *Semin Arthritis Rheum*. 2002;32:10-37.
- Coleman PJ, Scott D, Mason RM, Levick JR. Role of hyaluronan chain length in buffering interstitial flow across synovium in rabbits. *J Physiol*. 2000;526(pt 2):425-434.
- Scott D, Coleman PJ, Mason RM, Levick JR. Concentration dependence of interstitial flow buffering by hyaluronan in synovial joints. *Microvasc Res*. 2000;59:345-353.
- Jubb R, Piva S, Beinat I, Dacre J, Gishen P. Structure modifying study of hyaluronan (500-730 kDa, Hyalgan) on osteoarthritis of the knee. *Arthritis Rheum*. 2001;44(suppl):155.
- Pasquali Ronchetti I, Guerra D, Taparelli F, et al. Morphological analysis of knee synovial membrane biopsies from a randomized controlled clinical study comparing the effects of sodium hyaluronate (Hyalgan) and methylprednisolone acetate (Depomedrol) in osteoarthritis. *Rheumatology*. 2001;40:158-169.
- Guidolin DD, Ronchetti IP, Lini E, Guerra D, Frizziero L. Morphological analysis of articular cartilage biopsies from a randomized, clinical study comparing the effects of 500-730 kDa sodium hyaluronate (Hyalgan) and methylprednisolone acetate on primary osteoarthritis of the knee. *Osteoarthritis Cartilage*. 2001;9:371-381.
- Frizziero L, Govoni E, Bacchini P. Intra-articular hyaluronic acid in the treatment of osteoarthritis of the knee: clinical and morphological study. *Clin Exp Rheumatol*. 1998;16:441-449.
- Listrat V, Ayrat X, Patarnello F, et al. Arthroscopic evaluation of potential structure modifying activity of hyaluronan (Hyalgan) in osteoarthritis of the knee. *Osteoarthritis Cartilage*. 1997;5:153-160.
- Weiss C, Band P. Musculoskeletal applications of hyaluronan and hylan. Potential uses in the foot and ankle. *Clin Podiatr Med Surg*. 1995;12:497-517.
- Wobig M, Dickhut A, Maier R, Vetter G. Viscosupplementation with hylan G-F 20: a 26-week controlled trial of efficacy and safety in the osteoarthritic knee. *Clin Ther*. 1998;20:410-423.
- Altman RD, Moskowitz R. Intraarticular sodium hyaluronate (Hyalgan) in the treatment of patients with osteoarthritis of the knee: a randomized clinical trial. Hyalgan Study Group [published correction appears in *J Rheumatol*. 1999;26:1216]. *J Rheumatol*. 1998;25:2203-2212.
- Mensitieri ML, Ambrosio L. Viscoelastic evaluation for different knee osteoarthritis therapies. *J Material Sci*. 1995;6:130-137.
- Tamoto K, Nochi H, Tada M, et al. High-molecular-weight hyaluronic acids inhibit chemotaxis and phagocytosis but not lysosomal enzyme release induced by receptor-mediated stimulations in guinea pig phagocytes. *Microbiol Immunol*. 1994;38:73-80.
- Peluso GF, Perbellini A, Tajana F. The effect of high and low molecular weight hyaluronic acid on mitogen-induced lymphocyte proliferation. *Curr Ther Res*. 1990;47:437-443.
- Presti D, Scott JE. Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl (OH) radicals is dependent on hyaluronan molecular mass. *Cell Biochem Funct*. 1994;12:281-288.
- Tobetto K, Nakai K, Akatsuka M, Yasui T, Ando T, Hirano S. Inhibitory effects of hyaluronan on neutrophil-mediated cartilage degradation. *Connect Tissue Res*. 1993;29:181-190.
- Yasui T, Akatsuka M, Tobetto K, Hayaishi M, Ando T. The effect of hyaluronan on interleukin-1 alpha-induced prostaglandin E₂ production in human osteoarthritic synovial cells. *Agents Actions*. 1992;37:155-156.
- Lisignoli G, Grassi F, Zini N, et al. Anti-Fas-induced apoptosis in chondrocytes reduced by hyaluronan: evidence for CD44 and CD54 (intercellular adhesion molecule 1) involvement. *Arthritis Rheum*. 2001;44:1800-1807.
- Smith MM, Ghosh P. The synthesis of hyaluronic acid by human synovial fibroblasts is influenced by the nature of the hyaluronate in the extracellular environment. *Rheumatol Int*. 1987;7:113-122.
- Akatsuka M, Yamamoto Y, Tobetto K, Yasui T, Ando T. In vitro effects of hyaluronan on prostaglandin E₂ induction by interleukin-1 in rabbit articular chondrocytes. *Agents Actions*. 1993;38:122-125.
- Tobetto K, Yasui T, Ando T, et al. Inhibitory effects of hyaluronan on [14C]arachidonic acid release from labeled human synovial fibroblasts. *Jpn J Pharmacol*. 1992;60:79-84.
- Gotoh S, Onaya J, Abe M, et al. Effects of the molecular weight of hyaluronic acid and its action mechanisms on experimental joint pain in rats. *Ann Rheum Dis*. 1993;52:817-822.
- Synvisc [package insert]. Ridgefield, NJ: Genzyme Biosurgery; 2002.
- Hyalgan [package insert]. New York, NY: Sanofi Pharmaceuticals Inc; 2000.
- Supartz [package insert]. Tokyo, Japan: Seikagaku Corporation; 2001.
- Nimrod A, Greenman B, Kanner D, Landsberg M. BioTechnology General Corp. High molecular weight sodium hyaluronate. US Patent No. 4, 784,990. Nov 15, 1988.
- Orthovisc [package insert]. Woburn, Mass: Anika Therapeutics Inc; 2004.
- Lindqvist U, Tolmachev V, Kairemo K, Astrom G, Jonsson E, Lundqvist H. Elimination of stabilised hyaluronan from the knee joint in healthy men. *Clin Pharmacokinet*. 2002;41:603-613.
- Lindenhayn K, Heilmann HH, Niederhausen T, Walther HU, Pohlenz K. Elimination of tritium-labelled hyaluronic acid from normal and osteoarthritic rabbit knee joints. *Eur J Clin Chem Clin Biochem*. 1997;35:355-363.
- Knudson CB, Knudson W. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. *FASEB J*. 1993;7:1233-1241.
- Aviad AD, Houpt JB. The molecular weight of therapeutic hyaluronan (sodium hyaluronate): how significant is it? *J Rheumatol*. 1994;21:297-301.
- Lesley J, Hasall VC, Tammi M, Hyman R. Hyaluronan binding by cell surface CD44. *J Biol Chem*. 2000;275:26967-26975.
- Maheu E, Ayrat X, Dougados M. A hyaluronan preparation (500-730 kDa) in the treatment of osteoarthritis: a review of clinical trials with Hyalgan. *Int J Clin Pract*. 2002;56:804-813.
- Kelly MA, Goldberg VM, Healy WL, Pagnano MW, Hamburger MI. Osteoarthritis and beyond: a consensus on the past, present, and future of hyaluronans in orthopedics. *Orthopedics*. 2003;26:1064-1079.

P. C. Vitanzo and B. J. Sennett

Hyaluronans: Is Clinical Effectiveness Dependent on Molecular Weight?

52. Espallargues M, Pons JM. Efficacy and safety of viscosupplementation with hylan G-F 20 for the treatment of knee osteoarthritis: a systematic review. *Int J Technol Assess Health Care*. 2003;19:41-56.
53. Wang CT, Lin J, Chang CJ, Lin YT, Hou SM. Therapeutic effects of hyaluronic acid on osteoarthritis of the knee. A meta-analysis of randomized controlled trials. *J Bone Joint Surg Am*. 2004;86:538-545.
54. Lo GH, LaValley M, McAlindon T, Felson DT. Intra-articular hyaluronic acid in treatment of knee osteoarthritis: a meta-analysis. *JAMA*. 2003;290:3115-3121.
55. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. Viscosupplementation for the treatment of osteoarthritis of the knee. *Cochrane Database Syst Rev*. 2005;Apr 18:CD005321.
56. Wobig M, Bach G, Beks P, et al. The role of elastoviscosity in the efficacy of viscosupplementation for osteoarthritis of the knee: a comparison of hylan G-F 20 and a lower-molecular-weight hyaluronan. *Clin Ther*. 1999;21:1549-1562.
57. Allard S, O'Regan M. The role of elastoviscosity in the efficacy of viscosupplementation for osteoarthritis of the knee: a comparison of hylan G-F 20 and a lower-molecular-weight hyaluronan. *Clin Ther*. 2000;22:792-795.
58. Pritchard CH, Sripada P, Bankes P, Smith D, Schneider D. A retrospective comparison of the efficacy and tolerability of sodium hyaluronate and hylan G-F 20 in the treatment of osteoarthritis of the knee. *J Musculoskelet Res*. 2002;6:197-205.
59. Moskowitz R. Commentary on Pritchard et al comparative retrospective study of hylan G-F 20 and sodium hyaluronate. *J Musculoskeletal Res*. 2004;7:v-vii.
60. Brown DJ, Wood EV, Hannah HM, Rao VS, Teanby D. Prospective comparison of sodium hyaluronate and hylan G-F 20 in a clinical practice: comment on the concise communication by Martens [letter]. *Arthritis Rheum*. 2004;50:1697-1698.
61. Garcia B. A comparative study: pseudoseptic reactions to Synvisc and not Hyalgan injections. Presented at: Annual Meeting of the American Academy of Orthopaedic Surgeons; March 10-14, 2004; San Francisco, Calif.
62. Richardson M, Larsen KM, Moore KD, Droege L, Heald DC. Randomized safety and efficacy comparison of hylan G-F 20 and sodium hyaluronate in knee osteoarthritis: a safety distinction [letter]. *J Bone Joint Surg Am*. June 30, 2004. Available at: <http://www.ejbs.org/cgi/eletters/84/9/1619>. Accessed March 10, 2005.
63. Hamburger MI, Brandt L, Bennett R, et al. Retrospective comparative analysis of the safety and efficacy of intraarticular hyaluronan therapies in clinical practice. *Osteoarthritis Cartilage*. 2004;12:S146.
64. Karlsson J, Sjogren LS, Lohmander LS. Comparison of two hyaluronan drugs and placebo in patients with knee osteoarthritis. A controlled, randomized, double-blind, parallel-design multicentre study. *Rheumatology*. 2002;41:1240-1248.
65. Kirschner M, Marshall, D.A. double-blind randomized controlled trial comparing alternate forms of high molecular weight hyaluronan for the treatment of osteoarthritis of the knee. *Osteoarthritis and Cartilage*. 2006;14:154-162.
66. ACR Subcommittee on Osteoarthritis Guidelines. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. *Arthritis Rheum*. 2000;43:1905-1915.
67. Hamburger MI, Lakhanpal S, Moar PA, Oster D. Intra-articular hyaluronans: a review of product-specific safety profiles. *Semin Arthritis Rheum*. 2003;32:296-309.
68. Bucher W, Otto T, Hamburger MI. Differentiation of hyaluronate products by qualitative difference in their immunogenicity in rabbits: possible mechanism for product-specific severe adverse reactions? *Arthritis Rheum*. 2002;46:2543-2544.
69. Balazs E, Leschiner A, Larsen N, Band P, inventors; Biomatrix Inc, assignee. Hylan preparation and method of recovery thereof from animal tissues. US patent 5,099,013. 1992.
70. Schiavinato A, Finesso M, Cortivo R, Abatangelo G. Comparison of the effects of intra-articular injections of hyaluronan and its chemically cross-linked derivative (hylan G-F20) in normal rabbit knee joints. *Clin Exp Rheumatol*. 2002;20:445-454.
71. Goldberg VM, Coutts RD. Pseudoseptic reactions to hylan viscosupplementation: diagnosis and treatment. *Clin Orthop*. 2004;419:130-137.
72. Jones KB, Patel PP, Deyoung BR, Buckwalter JA. Viscosupplementation pseudotumor. A case report. *J Bone Joint Surg Am*. 2005;87:1113-1119.
73. Sasaki M, Miyazaki Y, Takahashi T. Hylan G-F 20 induces delayed foreign body inflammation in guinea pigs and rabbits. *Toxicol Pathol*. 2003;31:321-325.
74. Jubb RW, Piva S, Beinat L, Dacre J, Gishen P. A one-year, randomised, placebo (saline) controlled clinical trial of 500-730 kDa sodium hyaluronate (Hyalgan) on the radiological change in osteoarthritis of the knee. *Int J Clin Pract*. 2003;57:467-474.
75. Frizziero L, Pasquali Ronchetti I. Intra-articular treatment of osteoarthritis of the knee: an arthroscopic and clinical comparison between sodium hyaluronate (500-730 kDa) and methylprednisone acetate. *J Orthop Traumatol*. 2002;3:89-96.