

Longterm Effects of Intraarticular Hyaluronan on Synovial Fluid in Osteoarthritis of the Knee

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ABSTRACT. *Objective.* Intraarticular (IA) hylan injections constitute second-line therapy for osteoarthritis (OA) of the knee, but human studies suggesting a possible mechanism of action are lacking. We examined the effect of IA Hylan GF-20 injections on synovial fluid (SF) hyaluronan (HA) concentration, viscosity, and elasticity over a 6-month period in patients with mild to moderate OA of the knees.

Methods. Patients with symptomatic knee OA (Osteoarthritis Research Society International grade 1–2) had SF aspirated from the study knee pre- and 3 and 6 months post-Hylan injection. Primary endpoints included SF HA concentration, viscosity, and elasticity. SF HA concentration was determined using uronic acid assay, and rheology measured using a micro-Fourier rheometer.

Results. Sequential SF samples were available from 32 of 60 subjects injected at baseline (15 men, 17 women; mean age 65 yrs) at 3 months post-injection. The mean HA concentration had increased by 13% ($p < 0.0008$), and the complex shear modulus had increased by 16% ($p < 0.03$). Sufficient SF was also available from 19 of these subjects at 6 months post-injection when mean HA concentration was 2.24 ± 0.62 mg/ml compared to their baseline mean of 2.02 ± 0.52 mg/ml, an increase of 10% ($p < 0.053$).

Conclusion. This open-label study showed a statistically significant change from baseline in both SF HA concentration and complex shear modulus at 3 months following IA Hylan GF-20 injection among subjects with mild to moderate knee OA. These results suggest that one possible mechanism of action of viscosupplementation is to promote endogenous HA production. Longer-term studies are required to identify whether these changes in SF measures are important for modification of disease progression in knee OA. (J Rheumatol 2006;33:946–50)

Key Indexing Terms:

HYALURONAN

OSTEOARTHRITIS

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RHEOLOGY

Osteoarthritis (OA) is a chronic disease characterized by gradual degradation of cartilage and failure of supporting joint tissues. It accounts for considerable morbidity in our community, with data from the Framingham study suggesting that symptomatic knee OA occurs in 6.1% of adults age 30 and over and symptomatic hip OA in 0.7%–4.4% of US adults¹. Current treatment strategies focus on symptomatic relief with paracetamol, nonsteroidal antiinflammatory drugs, nutraceuticals, and intraarticular (IA) agents, including glucocorticoids and hyaluronans. The American College of Rheumatology guidelines for the medical management of OA of the knee recommend use of IA hyaluronan as second-line therapy for the treatment of this condition²; however, there are few human studies of possible mechanisms of action of these agents. One rationale for the use of these agents relates to the impairment of synovial fluid (SF) viscosity and elasticity in patients with

OA. This is primarily due to the decrease in native hyaluronan (HA) concentration and molecular weight^{3,4}. It has been postulated that administration of IA HA improves joint rheology by increasing SF elasticity and viscosity, although there is very little evidence to support this. A study by Grecomoro, *et al*⁵ demonstrated a short-term restoration in SF viscosity at 3 weeks post-administration of high molecular weight IA HA, although no statistical analysis was provided. A study by Mensitieri, *et al*⁶ reported a 20% improvement in SF viscosity and elasticity in the HA group compared with placebo, but no statistical analysis was provided and followup was only short-term.

We evaluated the longterm (3 and 6 months) effects of 3 weekly injections of Hylan GF-20 (Synvisc) on the SF parameters HA concentration and viscoelasticity. Ethics approval for the study was obtained from the Royal North Shore Hospital Human Research Ethics Committee, and informed consent was obtained from all study recruits.

MATERIALS AND METHODS

Patients. Patients were recruited through referrals from visiting rheumatologists to the Royal North Shore Hospital and advertisements in local newspapers. Sample size estimates suggested that 30 subjects with sequential SF samples would be sufficient to detect a 10% change from baseline in the primary endpoint, SF parameters.

Inclusion criteria. Men or women aged between 40 and 80 years; with radiological OA grades 1 to 2 (Osteoarthritis Research Society International) on a

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semiflexed fluoroscopically controlled weight-bearing radiograph of the knees; and with no IA steroid injections in the preceding 3 months and no form of viscosupplementation in the previous 12 months. Study subjects were asked to discontinue any supplements containing glucosamine and/or chondroitin sulfate at least 1 month prior to being screened and to remain off these agents for the duration of the study. Subjects were able to continue taking stable doses of any antiinflammatory or analgesic medications.

Inclusion criteria also specified that subjects were only considered for participation upon aspiration of at least 0.2 ml SF at baseline and at 3 months post-Hylan injection. Failure to aspirate SF at 6 months did not result in exclusion from analysis. Significant difficulties in meeting the criteria for SF aspiration at baseline and at 3 months post-injection led to the use of ultrasound guided joint aspirations toward the latter part of the study.

Exclusion criteria. Those with known underlying inflammatory arthritis or crystal arthropathy, or with large clinically significant synovial effusions (estimated > 10 ml) were excluded from the study, as they may have represented an undiagnosed inflammatory process. Only one patient was excluded for this reason.

Synovial fluid collection. SF was collected from each study recruit by the same investigator and transported immediately to the laboratory on ice; the samples were centrifuged for 10 min and the supernatant pipetted into 1.5 ml Eppendorf tubes and stored at -80°C until analysis. The samples were then thawed and analyzed in 4 batches of 8 patients each over a 6-month period using methods listed below. Samples were coded such that the investigator performing the analyses was blinded to subjects' names and sequence of sample collection.

Hyaluronan concentration. The hyaluronan concentration of the SF samples was determined by a modified micro-method of the uronic acid assay by Blumenkrantz and Asboe-Hansen⁷. The diluted SF samples were boiled with sulfuric acid/borax and a pink color developed by the addition of m-phenylphenol. The color intensity was then determined in a microtiter plate reader and compared with that of known standard concentrations of commercially available HA preparations. All assays were performed with triplicates of each sample to reduce error, and the mean of the replicates was used for analysis.

Rheology measurements. SF rheology was determined using a micro-Fourier rheometer. A small amount of fluid is placed on a metal plate, which is then subject to compression over a range of experimental frequencies ranging from 0 to 20 Hz. All samples were run against a commercial standard and results calibrated accordingly. Using a computer, a Fourier conversion is applied. The data were expressed as complex shear modulus (CSM)

$$G^*(\omega) = \sqrt{G'^2 + G''^2}$$

The rheometer expressed values of G' (elasticity) and G'' (viscosity) at 0.2-Hz intervals between 0 and 20 Hz. We used values at 1 Hz for all calculations, although the significances of the results did not differ whether calculations were done at 0.5, 1, or 10 Hz.

Statistical analysis. All results were entered into an Excel spreadsheet and then exported into Stata statistical software. Changes in HA concentration and CSM pre- and post-Hylan injection were calculated using the Wilcoxon signed-rank test and paired Student *t* test. Correlations were determined using Spearman's correlation coefficient.

RESULTS

Baseline. A total of 60 patients were recruited to yield 32 patients [15 men and 17 women, mean age 65 yrs (range 42–87 yrs) and mean body mass index (BMI) 29 kg/m² (range 23–40 kg/m²); Table 1] who satisfied the inclusion criteria, giving at least 0.2 ml SF at baseline and 3 months post-Hylan injections.

Of the 28 subjects (13 men, 15 women) who did not meet

Table 1. Baseline characteristics by sex. Values are mean (\pm SD). There were no statistically significant differences in any baseline measures between male and female subjects.

Characteristics	Men	Women
No.	15	17
BMI, kg/m ²	29.4 (\pm 4.9)	28.7 (\pm 4.9)
Minimal JSN, mm	3.8 (\pm 2.0)	3.2 (\pm 1.2)
Synovial fluid volume, ml	3.6 (\pm 3.7)	4.2 (\pm 4.2)
WOMAC pain (per 100)	34 (\pm 21.1)	44 (\pm 18.0)
WOMAC function (per 100)	43 (\pm 21.8)	49 (\pm 18.9)
Age, yrs	64 (\pm 11.5)	66 (\pm 9.5)
HA concentration, mg/ml	2.1 (\pm 0.7)	1.9 (\pm 0.6)
Complex shear modulus, Pa	2.4 (\pm 2.4)	1.7 (\pm 1.9)

BMI: body mass index, JSN: joint space narrowing, WOMAC: Western Ontario and McMaster Universities OA index, HA: hyaluronan.

the SF inclusion criteria, the mean age was 56 years (range 43–78) and BMI was 30.5 kg/m² (range 24–44). There was no statistically significant difference of mean BMI, mean baseline pain score on visual analog scale (VAS), or radiographic severity between these subjects and the 32 subjects with analyzable data. They were, however, significantly younger and the 5 subjects who had sufficient SF at baseline, but not followup, had a higher mean baseline HA concentration (2.29 mg/ml). There was a good correlation between baseline HA concentration and CSM ($r = 0.59$, $p < 0.0005$; Figure 1, Figure 2). There was no significant relationship between the changes in HA concentration and baseline values, and there was no significant correlation between changes in HA concentration and CSM at 3 months. Mean total SF volume of aspiration at baseline and 3 months was 3.91 ml and 3.89 ml, respectively, with no statistically significant differences at the 2 timepoints. Similarly, there was no significant difference in SF total white blood cell count at baseline and at 3 months, with mean values of $250 \times 10^6/l$ and $300 \times 10^6/l$, respectively.

3 and 6 months post-injection. At 3 months post-injection, 27 subjects had sustained an increase, and 5 a decrease, in SF HA concentration. The mean HA concentration had increased by 13% ($p < 0.0008$; Table 2).

At 6 months, there were only 19 subjects who had sufficient SF for HA concentration analysis. Of the 27 subjects that had sustained an increase at 3 months, 14 remained above baseline at 6 months, 5 had fallen to baseline levels or below, and 8 had insufficient SF to perform the analysis.

The mean HA concentration of the 19 subjects at 6 months post-injection was 2.24 ± 0.62 mg/ml compared to their baseline mean of 2.02 ± 0.52 mg/ml, an increase of 10% ($p < 0.053$) that was not significant.

Of the 32 subjects with analyzable data, 22 had sustained an increase and 10 a decrease in CSM 3 months post-injection. The mean change at 3 months was an increase of 16% ($p < 0.03$; Table 2). At 6 months, only 18 subjects had sufficient SF to perform rheology measurements; one subject had

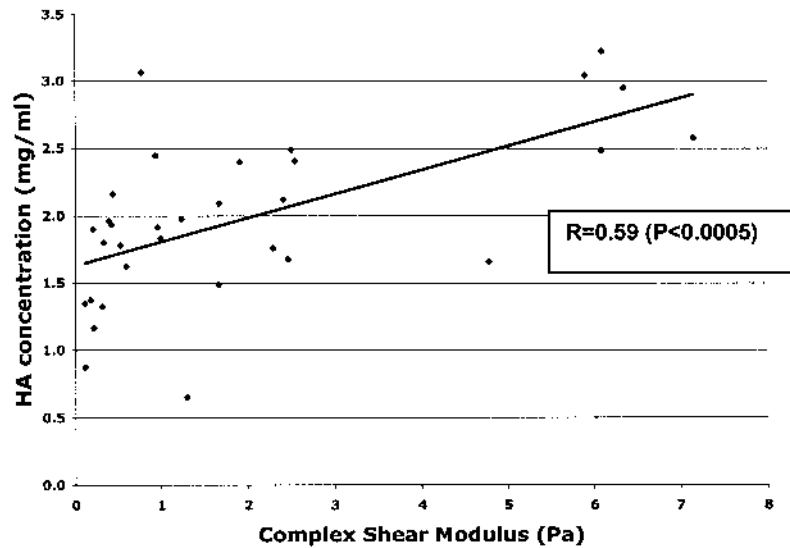


Figure 1. Relationship between baseline synovial fluid hyaluronan (HA) concentration and complex shear modulus.

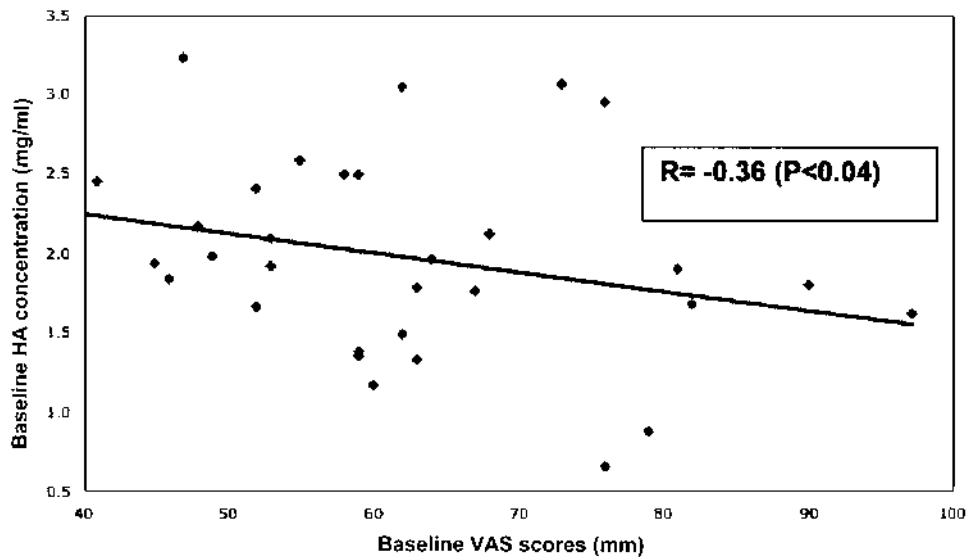


Figure 2. Relationship between baseline hyaluronan (HA) concentration and pain scores on visual analog scale (VAS).

only enough SF for HA concentration measurements but not CSM measurements. The mean CSM among these subjects at 6 months was 1.80 ± 1.95 Pa. Compared to the baseline mean of these 18 subjects of $1.75 (\pm 1.56)$ Pa, the change in CSM was not significant ($p < 0.56$).

DISCUSSION

We demonstrated that a single course of 3 weekly intraarticular Hylan injections into knees of patients with mild to moderate OA has a statistically significant influence on synovial fluid hyaluronan concentration 3 and 6 months post-injection, and results in a significant improvement in the viscoelasticity

of SF at 3 months. Given the known residence time of Hylan GF-20 in the knee of 8.8 days⁹, this would suggest a stimulation of endogenously produced HA and may suggest potential for altering disease progression. Evidence for stimulation of native HA production with administration of exogenous HA was demonstrated by Smith and Ghosh in 1987¹⁰. In that study, synovial fibroblasts derived from an OA joint showed the most marked response on exposure to exogenous HA, showing a stimulation of HA synthesis with preparations of average molecular weight greater than 5×10^5 in a concentration-dependent manner. However, this may not be directly comparable to Hylan GF-20, which has a much higher molec-

Table 2. Synovial fluid measures at baseline and 3 months. Data are mean (\pm SD).

	Baseline	3 Months	Change
No. of patients with sufficient SF to meet inclusion criteria	32	32	—
Mean HA concentration, mg/ml	1.97 (\pm 0.61)	2.22 (\pm 0.63)	\uparrow 13% $p < 0.0008$
Mean complex shear modulus, Pa	1.99 (\pm 2.1)	2.30 (\pm 2.3)	\uparrow 16% $p < 0.03$

ular weight (6×10^6). In another study of 7 patients with knee OA, a single injection of Healon was administered intraarticularly: HA concentration was found to be increased in 6 of the 7 patients by a mean of 15%, an average of 10 days post-injection¹¹. The longest period post-injection for SF aspiration was 22 days, and 4 patients who had viscosity measured pre- and post-injection all demonstrated an increase. No statistical measure was provided in that study, but both studies suggest a potential for stimulation of endogenous HA production with exogenous HA preparations, and also suggest a possible mechanism of action of this class of agents. We were able to observe a modest but sustained increase in HA concentration at 3 and 6 months post-Hylan injection.

The viscoelastic characteristics of SF in the joint are entirely due to its HA content. At low frequency SF demonstrates very high viscosity, and at higher frequencies, the fluid becomes highly elastic. In OA, the elasticity and viscosity of SF are much lower than in the normal joint, and this is primarily due to a marked decrease in HA concentration and molecular weight³. It is hypothesized that this compromise in viscoelasticity leads to the diminished rheological properties and molecular barrier effects normally imparted by HA, and leads to unmitigated forces transmitted to the cartilage and intercellular matrix. During human walking, the strain frequency on SF in the knees is in the range of 0.1–0.5 Hz, and during running and jumping, strain frequency increases to 2.5 Hz¹². In normal human adult knees, the SF HA begins to change from a predominantly viscous solution to an elastic solution at a strain frequency of around 0.5 Hz¹². The strain frequency at which the G' and G'' intersect is referred to as the crossover point, and in OA SF the crossover point occurs at much higher strain frequencies than in normal joint fluid, or in some individuals the curves do not cross at all. We chose to use a composite measure of both G' (elasticity) and G'' (viscosity) — the complex shear modulus represented by the equation shown above⁸.

We chose 1 Hz as the single strain frequency to measure response, given it represents the transition between walking and running. Results were calculated at 0.5 Hz and 10 Hz, and the significance of response to Hylan injections did not change. The mean CSM among the 32 subjects increased by 16% at 3 months ($p < 0.02$), but at 6 months only 18 subjects had sufficient SF for aspiration. Among these 18, the mean

baseline CSM had fallen back to pretreatment levels, with no statistically significant difference from baseline. We were unable to assess change in crossover points pre- and post-treatment as measurements in strain frequency below 0.1 Hz had too much noise due to interference from very low frequency vibrations within the laboratory.

HA concentration in the SF of a healthy adult knee resides in the range of 2–2.5 to 4 mg/ml, whereas in OA knees HA concentration falls to 1–2 mg/ml. Similarly, values of elasticity (G') and viscosity (G'') in normal and OA SF at 2.5 Hz are 23 Pa and 8 Pa, falling to 7 Pa and 5 Pa, respectively. In our study the mean HA concentrations at baseline were 1.98 mg/ml (range 0.65–3.23 mg/ml), and while the absolute mean increases in HA concentration 3 and 6 months post-injection were modest, they had risen to the lower range of values for adult healthy knees.

There are several limitations to this study. We acknowledge that low-grade inflammation is a common finding in patients with OA, and by requiring at least 0.2 ml of fluid at baseline and followup we may have been selecting a group more likely to have low-grade inflammation. We did exclude one patient with a clinically significant synovial effusion (> 10 ml) on the basis that those with large effusions may be more likely to have crystal arthropathy or some other inflammatory arthritis. Previously published efficacy studies with Hylan had also excluded such patients.

The most significant limitation is the lack of a formal control group. The results of this pilot study do, however, provide important parameters for planning any future controlled trials to evaluate whether HA concentrations in SF vary over time with no intervention or equally with aspiration alone or administration of normal saline or other products intraarticularly. However, a recent study by Miyaguchi, *et al*¹³ looked at the effect of isometric quadriceps strengthening on SF measures; they found that SF HA concentration remained statistically significantly unchanged in both the intervention and the control group over a 12-week period. The percentage change in HA concentration over 12 weeks in the intervention group was less than 1%. By comparison, the 13% change in our study is likely to indicate both a statistically and a clinically meaningful change. As well, the initial work by Balazs, *et al*³ suggests that HA concentration remains stable over many months in the absence of any specific intervention. Further limitations in this study include the lack of data to address other factors that can influence SF variables, including rate of ingress and egress of water and total HA volume in the knee at the time of aspiration. These factors are not easy to control for, and the only data we have is total mean SF volume at both timepoints — 3.91 ml at baseline and 3.89 ml at 3 months, with no significant difference. Subjects with clinically significant effusions were excluded from the study, as it was thought the presence of inflammation would have a significant effect on SF measures.

This is the largest prospective study of SF measures over a

6-month period after administration of IA Hylan GF-20. We observed a statistically significant improvement in both SF HA concentration and complex shear modulus at 3 months post-administration of IA Hylan GF-20 in subjects with mild to moderate knee OA. These results suggest that one possible mechanism of action of viscosupplementation is to promote endogenous HA production. It remains to be determined whether this rise in SF HA concentration will translate to longer-term benefits for joint structural integrity; however, these preliminary data would suggest it is worthy of further study.

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