Amelioration of Disease Severity by Intraarticular Hylan Therapy in Bilateral Canine Osteoarthritis


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Summary: Because of its high molecular weight, the glycosaminoglycan molecule hyaluronan is responsible for the viscoelastic properties of normal synovial fluid. In osteoarthritis, the concentration and molecular weight of hyaluronan in synovial fluid is diminished; this impairs the ability of synovial fluid to effectively lubricate joints, absorb loads, and exert anti-inflammatory effects. Using a bilateral anterior cruciate-ligament transection and partial neurectomy canine model of osteoarthritis, this study examined the effect of viscosupplementation with hylan G-F 20 as a treatment for osteoarthritis. Twelve dogs underwent bilateral arthroscopic anterior cruciate-ligament transections and partial neurectomy of the knee joints. Beginning 1 week after the operation, six dogs received three weekly 500-μl injections of hylan G-F 20 in one knee and a sham injection of saline solution in the contralateral knee (early-treatment group). The remaining six animals underwent the same treatment 2 months following the procedure (late-treatment group). All dogs were killed at 8 months, and both knees were evaluated for gross pathology, histology, and proteoglycan content. In addition, with use of 500-MHz [1H] magnetic resonance spectroscopy, the synovial fluid from both knees was assessed for changes in metabolic profile. Differences in outcome were analyzed with paired t tests. Gross pathological and histological examination revealed significantly less severe changes of osteoarthritis in knees treated with hylan G-F 20 2 months after surgery than in the contralateral untreated knees. Magnetic resonance spectroscopy of the specimens in this late-treatment group showed significantly decreased glucose concentrations and significantly elevated isoleucine levels in the synovial fluid from knees treated with hylan G-F 20 compared with the controls. Previous magnetic resonance spectroscopy had shown that glucose concentrations increase with the onset of osteoarthritis and eventually diminish in end-stage osteoarthritis. The three injections of hylan were given after osteoarthritis was established, and the severity of the disease was ameliorated in the treated knees 6 months after treatment. This occurred although hylan G-F 20 is almost certainly cleared from joints by lymphatics within 4 weeks of injection, suggesting that hylan therapy can retard the progression of osteoarthritis for periods of time extending beyond the intraarticular residence time of the injected molecules and that hylan injections given at relatively early stages of osteoarthritis may have a chondroprotective effect. No changes in outcome were noted in the animals that received hylan G-F 20 immediately following surgery.

Osteoarthritis is the most prevalent age-related disease in humans (16,26). Traditional treatment modalities have targeted relief of symptoms rather than modification or cure of the disease. These therapies include analgesics, nonsteroidal anti-inflammatory drugs, intraarticular steroids, arthroscopic debridement, joint replacement, physiotherapy, and walking aids.

Hyaluronan is a negatively charged, extremely hydrophilic glycosaminoglycan, which, because of its high molecular weight, is entirely responsible for the viscoelastic properties of normal synovial fluid (49). These properties are crucial for the proper maintenance of joint homeostasis. Hyaluronan lubricates joint surfaces, helps dissipate loads transmitted across articular surfaces, limits migration of inflammatory cells to articular cartilage, and provides protection for articular pain receptors and synoviocytes (10,23,29,42,43,45).

In osteoarthritis, the concentration and molecular weight of hyaluronan in synovial fluid is markedly diminished (7,8). The concentration of hyaluronan is decreased by dilution secondary to exudation. The size of hyaluronan molecules decreases due to fragmentation of hyaluronan in the synovial fluid or production of abnormally small hyaluronan by native synoviocytes, or both. These changes diminish the vis-
coelasticiiy of the synovial fluid and impair the joint homeostatic functions of hyaluronan.

Intraarticular hyaluronan therapy has been advocated as a treatment for osteoarthritis for almost 2 decades (9,14,43,44,47). However, the efficacy of this approach and its place in the osteoarthritis treatment armamentarium remain equivocal. This may be because the MW of the hyaluronan products available for human joint injection has heretofore been relatively low (range: 500,000-750,000); therefore, they do not adequately restore the rheological properties of osteoarthritic synovial fluid to normal levels (normal synovial fluid MW: 5 million). In addition, exogenous hyaluronan is quickly cleared from joints by lymphatics. To address these limitations, a new class of crosslinked high-molecular-weight hyaluronans, called hylans, has been developed (10,11,54). Like hyaluronans, hylans have lubrication, load transmission, anti-inflammatory, and antinociceptive properties that can help restore and maintain joint homeostasis. Hylan G-F 20, a hylan that has a high MW (6 million) and increased joint-residency time compared with the low-molecular-weight hyaluronan preparations, has become clinically available.

In vitro and in vivo evidence suggests that treatment based on hyaluronan can be chondroprotective. Hyaluronan stimulates the production of tissue inhibitors of metalloproteinases (TIMPs) by cultured chondrocytes (56). A higher-molecular-weight hyaluronan more effectively stimulated the production of TIMP-1 than did a lower-molecular-weight preparation. In addition, in a viscosity-dependent fashion, hyaluronan inhibited neutrophil-mediated cartilage degradation (50). Lastly, chondrocyte injury and matrix degeneration in cartilage explants subjected to interleukin-1 (IL-1), degradative enzymes, and oxygen-derived free radicals were attenuated by treatment with hylan (28).

In this instance, high-molecular-weight hylan had a greater protective effect on cartilage than did the lower-molecular-weight hyaluronan.

Data from animal models of arthritis also suggest that hyaluronan-based treatment can ameliorate the progression of osteoarthritis. Rabbit knees treated with hyaluronan had a decrease in the severity of osteoarthritic change 4 weeks after anterior cruciate-ligament transection compared with controls (25), and higher-molecular-weight hyaluronan was more effective than lower-molecular-weight hyaluronan. Treatment with hylan in the rabbit model of severe-instability osteoarthritis delayed the onset and decreased the severity of cartilage degeneration in animals studied to 16 weeks (53).

In a milder form of experimental osteoarthritis in the rabbit, higher-molecular-weight hyaluronan had a greater protective effect on cartilage than did lower-molecular-weight hyaluronan (57). In the model of meniscectomy in sheep, five weekly injections of hyaluronan significantly decreased the osteoarthritic changes in articular cartilage and subchondral bone (5). Hyaluronan also retarded the severity of induced osteoarthritis in the canine model of unilateral anterior cruciate-ligament transection (1).

In recent years, the bilateral arthroscopic anterior cruciate-ligament transection model of osteoarthritis produced symmetrical osteoarthritis in the knee joints of dogs (34,35). Furthermore, articular nerve injury accelerated the rate of development of osteoarthritis in the canine anterior cruciate-ligament transection model (40). With this approach, symmetrical osteoarthritis was established in the knees by 2 months after bilateral anterior cruciate-ligament transections and partial joint denervations.

High-resolution [1H]magnetic resonance spectroscopy (MRS) has been used to study the synovial fluid in osteoarthritis knee joints of dogs and humans (17,18). Because the degradation products, enzymes, and signal-transduction molecules involved in osteoarthritis are first released from the cartilage matrix into the synovial fluid, analysis of joint fluid provides biochemical information about the metabolic state of a specific joint. MRS is one of the most powerful methods in analytical chemistry: it can observe and quantify a large number of endogenous metabolic species simultaneously and with the same sensitivity, making it well suited for the biochemical assessment of synovial fluid.

We have used high-resolution MRS to investigate and compare the metabolic profiles of normal and osteoarthritic synovial fluids in a canine model of osteoarthritis (18). Compared with healthy normal canine synovial fluid, the osteoarthritic synovial fluid had increased concentrations of lactate, pyruvate, lipoprotein-associated fatty acids, glycerol, and the ketones hydroxybutyrate and hydroxy-isobutyrate; reduced levels of glucose; and elevated levels of N-acetylglycoproteins, acetate, and acetylamide. The concentrations of the amino acids alanine and isoleucine were also increased in osteoarthritic synovial fluid.

These results suggest that (a) the intraarticular environment is more hypoxic and acidic in canine osteoarthritic than in the normal joint; (b) lipolysis may play an increasingly important role as a source of energy in osteoarthritis; and (c) the N-acetylglycoprotein polymer component of synovial fluid (mostly hyaluronan) seems to be increasingly fragmented and degraded into acetate by an acetamide intermediate with progressive osteoarthritis.

The hypothesis underlying this study is that viscosupplementation can have a chondroprotective effect on diseased or injured articular cartilage. The purpose of this study was 2-fold. First, it examined the effectiveness of viscosupplementation with three weekly
TABLE 1. Gross pathology scores for the dogs in the late-treatment group

<table>
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<tr>
<th>Dog</th>
<th>Limb</th>
<th>Medial femoral condyle</th>
<th>Lateral femoral condyle</th>
<th>Patellar groove</th>
<th>Medial tibial plateau</th>
<th>Lateral tibial plateau</th>
<th>Patella</th>
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Dogs treated with hylan G-F 20 had significant amelioration of osteoarthritis relative to the controls (p < 0.05). The mean (±SEM) total pathology score for the experimental limbs was 10.83 (1.47), and that for the control limbs was 13.50 (1.65). A curvilinear 15-cm incision was made beginning at the middle of the thigh and extending distally to the Achilles tendon. The interval medial to the hamstring muscles was utilized to identify the major neurovascular structures of the posterior knee. The tibial nerve was followed distally by dissecting in the plane between the gastrocnemius and soleus muscles. The posterior articular nerve was found on the lateral soleus muscle at the level of the knee joint. The nerve received a crush injury for 1 minute by a 75-g spring-loaded clip.

The animals were monitored for signs of pain, infection, anorexia, and weight loss in the postoperative period. The skin sutures were removed at 10 days. Barring complications, the dogs were transferred to outdoor kennels, allowing for spacious runs.

Materials and Methods
Preparation of the Animal Model
The protocol for the use of animal subjects was reviewed and approved by the Animal Care Committee of The Toronto Hospital. A sample-size calculation was performed with use of a power of 0.8, an alpha error of 0.05, a threshold of improvement of 2.0 on the scale of Mankin et al. (32), and the variability from data in previously performed validation studies of the bilateral anterior cruciate-ligament transection model of osteoarthritis in dogs (27). Six dogs per group were determined adequate to detect differences between the two groups. Thus, 12 mongrel adult dogs (age: >2 years; weight: 20-36 kg) were used in the study. The surgical procedures were performed under sterile conditions in animal operative suites (34,35).

Bilateral medial articular nerve injury: A 3-cm transverse incision was made on the medial aspect of the knee joint, starting over the medial epicondyle and extending posteriorly. The underlying fascia was incised and the neurovascular structures were readily identified under the dissecting microscope. The medial articular nerve received a crush injury for 1 minute with a 75-g spring-loaded clip.

Bilateral arthroscopic anterior cruciate-ligament transection: A 2.7-mm arthroscope (Dyonics, Andover, MA, U.S.A.) at a 30° angle was interfaced with a fiberoptic light source (34,35). The anterior cruciate ligament was cut with scissors under direct visualization with the arthroscope. The transection was confirmed by direct visualization and anterior drawer examination with the dogs under anesthesia.

Bilateral posterior articular nerve injury: On completion of the arthroscopy, the animals were transferred to the prone position, and intraarticular injections of hylan G-F 20 in ameliorating the progression of established mild osteoarthritis in the accelerated bilateral canine osteoarthritis model. The second purpose was to assess the ability of hylan to prevent the development of osteoarthritis in the same model. Methods of assessment included gross pathological and histopathological examination, biochemical analysis, and MRS of synovial fluid.

Hylan Injections
Six animals underwent a series of three injections a week beginning the week after surgery (early-treatment, osteoarthritis-prevention group). A second group of six dogs received a series of three injections beginning 8 weeks after surgery (late-treatment, osteoarthritis-amelioration group). The animals were premedicated with a cocktail of Acraven (acepromazine), Demerol, and atropine. A 21-gauge needle was introduced into both knee joints, and aspiration of synovial fluid with a 5-ml syringe confirmed position in the joint space. A 500-μl (4 mg) injection of hylan G-F 20 (Synvisc; Biornatrix, Montreal, Canada) was then administered in the treated knee, and a 500-μl sham injection of normal saline solution (Baxter, Deerfield, IL, U.S.A.) was given in the control knee.

Gross Examination
At death, the knees were retrieved from the cadavers, disarticulated, and examined. Each knee was examined for the presence of osteophytes, the condition of the menisci, and the state of the articular cartilage overlying the femoral condyles, tibial plateaus, femoral groove, and patella. Gross osteoarthritic changes were graded on a scale from 0 to 4 as follows: 0 = normal, 1 = patches of fibrillation or softening, 2 = more pronounced fibrillation with early marginal chondroosteophytosis and related synovial hyperplasia, 3 = commencing subarticular bone exposure, more generalized synovial disease, and osteophytes, and 4 = extensive cartilage loss and bone exposure with chondral and bone groove.
TABLE 2. Gross pathology scores for the dogs in the early-treatment group

<table>
<thead>
<tr>
<th>Dog, limb</th>
<th>Medial femoral condyle</th>
<th>Lateral femoral condyle</th>
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<th>Medial tibial plateau</th>
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The mean (±SEM) total pathology score for the experimental limbs was 7.67 (1.23), and that for the control limbs was 8.83 (1.30); p > 0.05, experimental compared with control limbs.

**Histological Examination**

Samples of articular cartilage were obtained from standardized areas on each of the six articular surfaces as recommended by Adams and Pelletier (2). With use of a biopsy trochar, 2-mm-diameter osteochondral plugs were obtained from the central weight-bearing areas of both femoral condyles and tibial plateaus, as well as from the patellar groove and the patella of all knees. The tissue was fixed in neutral buffered 10% formalin until batch processing. The samples were then decalcified and embedded in paraffin. A microvome was used to cut 7-µm sections, which were then mounted on glass slides and stained with safranin O and toluidine blue (46). Four sections were cut and mounted from each of the six articular surfaces. The histology slides were scored with a modification of the method of Mankin et al. (32). The severity of surface fibrillation, chondrocyte clustering, and superficial chondrocyte loss was graded. For fibrillation, 0 = absent, 1 = minimal fibrillation, 2 = fibrillation into the superficial third of cartilage thickness, and 3 = complete cartilage loss. For clustering, 0 = normal, 1 = clusters in the superficial layer, 2 = clusters in the deep layers, and 3 = complete loss of cartilage. For loss of superficial chondrocytes, 0 = no loss and 1 = loss. Furthermore, every other slide was stained with toluidine blue. Proteoglycan content was assessed by determining the degree of loss by metachromatic staining: 0 = no loss, 1 = loss from the upper third of cartilage depth, 2 = loss extending into the middle third of the samples, and 3 = complete loss of cartilage. All samples were scored by a pathologist who was blinded to which knee was experimental and which was control. A cumulative score was calculated for each knee. The experimental and control knees for each treatment group were subsequently pooled and compared with paired t tests.

**Assay of Proteoglycan Content with Alcian Blue**

The content of sulfated proteoglycan in the cartilage samples was determined with an alcian blue (Sigma Chemical, St. Louis, MO, U.S.A.) precipitation assay (27,37). Cartilage samples, 5-15 mg, were digested overnight in a 0.5 M solution of sodium acetate, pH 6.8, with 10 U/ml papain (Boehringer, Mannheim, Germany). Fifty-microgram (100 µl) samples of cartilage were mixed with 150 µl of a 0.2% alcian blue/0.12% MgCl₂ solution. Following centrifugation, the precipitated pellet was washed and then suspended in a 2% sodium dodecyl sulfate solution. A spectrophotometer measured the optical density of the solutions at 620 nm. The concentration of sulfated proteoglycans was then calculated by comparing measurements with the linear portion of a standard curve prepared with shark chondroitin sulfate.

**Collection and Preparation of Synovial Fluid**

Paired samples of synovial fluid were obtained (8 months after the operation) from the experimental (treated with hylan) and contralateral control (treated with saline solution) osteoarthritic knees from each animal at death. We consistently retrieved 0.3-1.0 ml of synovial fluid aspirate. We did not have to inject saline solution for dilution and retrieval of the fluid. All samples were centrifuged at 14,000 rpm (14,000 × g) for 15 minutes to remove particulate matter and cells. They were subsequently made up in a ratio of 420 µl of synovial fluid mixed with 280 µl D₂O. Six hundred microliters of the mixture was placed into 5-mm thin-walled nuclear magnetic resonance (NMR) tubes (Wilmad Glass, Buena, NJ, U.S.A.).

Chemical shifts of all observed metabolites were referenced to a known-concentration (0.5 mg/100 µl) external-internal standard of sodium 3-(trimethylsilyl-2,2,3,3,3,H₄)-1-propionate (TSP) (δ = 0 ppm) contained inside a capillary tube placed coaxially at the centre of each NMR tube.

**Proton MRS**

Data were acquired on a spectrometer (Varian 500; Carbohydrate Research Center-Nuclear Magnetic Resonance Facility, Medical Science Building, University of Toronto, Toronto, Ontario, Canada) operating at 500.188 MHz 1H with the Carr-Purcell-Meiboom-Gill (15.58) pulse sequence D = 90°, τ = (180°, - τ), - acquire, where D = 3 seconds, τ = 1 millisecond, and n was set so that the total T2 relaxation decay 2 m = 48 milliseconds and the acquisition time T1 = 1.333 seconds. The echo delay time τ was chosen to suppress the broad protein background by using its short spin-spin relaxation time T2 while retaining substantial lipid resonance intensity. In the pulse sequence, τ refers to the axis in the rotating frame of nuclear spins, about which the first pulse of the sequence is applied; the τ refers to the axis along which echoes form. All spectra were measured at ambient probe temperature.
(25°C). For each sample, 256 free induction decays (FIDs) were acquired into 16,384 computer data points with a spectral width of 6,000 Hz.

The intense water signal was suppressed by applying a gated secondary irradiation field at the water resonance frequency during the delay between pulses. Resonance assignments were made on the basis of published values of chemical shifts in biological fluids (12,13,21,22,39).

Quantitative spectral analysis was performed using the SA/GE analysis software (General Electric Medical Systems, Waukesha, WI, U.S.A.) with a computer routine developed at our site. After phasing and baseline offset correction, the zero of chemical shift was set at the maximum of the TSP peak and the spectrum was scaled so that the amplitude of the TSP peak was equal to one.

Quantitation was obtained through a combination of integration and Marquardt-Levenberg (30,33) line-fitting routines. With the SA/GE peak-picking/integration routine and the fact that the Lorentzian wings of a given resonance were minimised by a Lorentz-Gauss transformation, the areas of all resolved resonances could be accurately measured. Where overlap occurred, peak areas were measured with the Marquardt-Levenberg Lorentz-Gaussian line-fitting routine. To account for variations between samples and experimental setups, all measurements were normalised with respect to the TSP reference area.

Statistical Analyses

Gross pathological examination: All data passed a normality test before analysis. Paired t tests were used to compare the gross pathological scores for the pooled experimental and control knee joints for each of the two treatment groups.

Histological examination: All data passed a normality test before analysis. Paired t tests were used to compare overall histological scores and separate scores for fibrillation, chondrocyte clustering, superficial chondrocyte loss, and metachromasia for the pooled experimental and control knee joints for each of the two treatment groups.

Proteoglycan content: All data passed a normality test before analysis. Paired t tests were used to evaluate differences in proteoglycan content between the six regions examined for the pooled experimental and control knee joints for each of the two treatment groups.

MRS: All data passed a normality test before analysis. Paired t tests were used to compare metabolite differences between the experimental and control knees of each animal for each of the two treatment groups.

All statistical analyses were performed on a pentium 166-MHz-based computer with SPSS software (version 7.0; Jandel Scientific, San Rafael, CA, U.S.A.) for Windows 95.

RESULTS

All dogs were fully weight-bearing within a few hours after the operation. At suture removal (10 days after the procedure), they were walking in the animal...
HYLAN THERAPY AMELIORATES CANINE OSTEOARTHRITIS

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FIG. 2. Toluidine blue-stained sections from the patellar groove of the control (A) and hylan-treated (B) knees of animal no. 2. Note the loss of purple metachromatic stain in the superficial cartilage and the extensive surface fibrillation in the control knee compared with the normal appearance of the knee treated with hylan (×10 magnification).

facility without any appreciable pain or limp. There were no infections or wound dehiscences after the operation, no infections, inflammatory complications, or other abnormalities after the intraarticular injections to either limb, and no gait or other medical problems during the dogs' stay in the outdoor kennels. At death, no appreciable limp was observed.

Gross Pathology

In all 24 knees, the anterior cruciate ligament was completely transected and the synovium was consistently and symmetrically hyperemic. The results for the dogs in the late (osteoarthritis amelioration) and early (osteoarthritis prevention) treatment groups are presented in Tables 1 and 2. Statistical analysis revealed that the overall score for arthritis severity in the knees injected with hylan in the late-treatment group was significantly decreased compared with that in the contralateral knees injected with saline solution (p < 0.05; paired t test). No significant differences were noted between the treated and control knees in the early-treatment group.

Histopathology

Figure 1 presents the histological data from the experimental and control knees for the two treatment groups. The experimental and control knees in the dogs in the early-treatment group did not differ significantly for any histologic parameter. For fibrillation and chondrocyte clustering, the knees treated with hylan in the late-treatment group had a statistically significant decrease in osteoarthritis severity compared with the contralateral knees injected with saline solution. Figure 2 illustrates typical histologic differences between articular cartilage treated with hylan and that treated with saline solution. For metachromasia, there was a trend (p = 0.076) toward decreased osteoarthritis severity in the knees treated with hylan in the
late-treatment group (Table 3). There was no difference between the experimental and control knees with respect to superficial chondrocyte loss in either the early or late-treatment groups. Table 3 presents an overall score for arthritis based on summation of the data acquired for the four histological indices for the late-treatment group; the total score of Mankin et al. was significantly decreased in the knees treated with hylan in this group.

**TABLE 3. Overall histological outcome for the dogs in the late-treatment group (mean ± SEM)**

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<td>Clustering</td>
<td>5.00 (±0.68)</td>
<td>6.33 (±0.42)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Metachromasia</td>
<td>0.84 (±0.40)</td>
<td>1.33 (±0.82)</td>
<td>0.076</td>
</tr>
<tr>
<td>Chondrocyte loss</td>
<td>6.0</td>
<td>6.0</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Total</td>
<td>15.17 (±1.40)</td>
<td>18.33 (±0.95)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

*Paired t test.

**Proteoglycan Content**

Each assay was performed in triplicate, and the average content was calculated. The six regions examined did not differ significantly in either the early or late-treatment group (data not shown).

**MRS Assessment of Synovial Fluid**

In the late-treatment group, the concentrations of MRS-determined β-glucose and other sugars/polyols in the synovial fluid were significantly decreased in the knees treated with hylan relative to the control knees whereas the level of the amino acid isoleucine was significantly elevated (both: p < 0.05) (Fig. 3). Levels of acetate and hydroxybutyrate tended to be decreased in the joint fluids of the knees treated with hylan (p < 0.1).

**DISCUSSION**

The recognition that synovial fluid hyaluronan has important functions in joint homeostasis, together with the observation that its quantity and character are changed in osteoarthritis, has led to the concept of viscosupplementation as a potential treatment for osteoarthritis. For longer than 20 years, this approach has been used to treat horses with posttraumatic arthritis. In recent years, interest in the use of hyaluronan to treat symptoms of osteoarthritis in humans has increased, fueled by accumulating evidence of the efficacy and safety of exogenous hyaluronans. A recurring theme for* in vitro* and *in vivo* animal studies is that as the MW of the hyaluronan preparations increases, its efficacy also increases (6,25,28,36,50,57). Most recently, a new class of crosslinked hyaluronans, hylans, has been developed; hylans have a much higher molecular weight and a prolonged intraarticular residence time compared with hyaluronans (10,11,54).

One rationale for the efficacy of viscosupplementation is that it improves joint mechanics and rheological properties. The nature of the intervention was originally thought to be temporary, because hyaluronan is quickly cleared from joints by lymphatics. However, it is becoming increasingly clear that the effects of viscosupplementation extend beyond a temporary improvement in joint rheological properties. Several additional mechanisms have been proposed to account for the prolonged symptomatic efficacy that hyaluronan/hylan injections provide to many patients with osteoarthritis. Normalization of hyaluronan synthesis by hyalocytes (51), direct anti-inflammatory ef-

**FIG. 3.** Average (± SEM) peak areas for significant components of hylan-treated and saline solution-treated (control) canine synovial fluid (late-treatment cohort) at a statistical significance level of p < 0.05.
fected (6,20,55), and antinociceptive effects (4,24,45) have been identified as possible mechanisms of symptom modification.

In this study, the knees treated with three hylan injections at a time point consistent with mild osteoarthritis (late-treatment group) demonstrated long-term (6 months) gross and histopathological amelioration of disease severity compared with the contralateral control knees. Several mechanisms have been identified that could underlie the hylan-mediated chondroprotection seen in the dogs in the late-treatment group. Hyaluronan can inhibit cartilage catabolic activity by stimulating the synthesis of TIMP-1 in chondrocytes (56). High-molecular-weight hyaluronan has been shown to be more effective than low-molecular-weight hyaluronan in stimulating the production of TIMP-1. Hyaluronan can also retard catabolic activity by inhibiting neutrophil-mediated cartilage degradation (50). Finally, chondrocyte injury and matrix degeneration in cartilage explants subjected to IL-1, degradative enzymes, and oxygen-driven free radicals were attenuated by viscosupplementation, and high-molecular-weight hylan had a greater protective effect than did the lower-molecular-weight hyaluronan (28).

Accumulating evidence from animal models of arthritis further suggests that viscosupplementation can ameliorate the progression of osteoarthritis in an anterior cruciate-ligament transection model of osteoarthritis in rabbits, osteoarthritis severity in knees treated with hyaluronan diminished at 4 weeks (25). Additionally, in a more severe rabbit model of osteoarthritis, hylan therapy delayed the onset and decreased the severity of cartilage degeneration at 16 weeks (53). In an ovine model of osteoarthritis, five weekly injections of hyaluronan retarded osteoarthritic changes in articular cartilage and subchondral bone (5). Previous work with hyaluronan therapy in experimental unilateral canine osteoarthritis also diminished the severity of osteoarthritis (1). However, the degenerative changes rapidly progressed when treatment was stopped.

Previous high-resolution [1H]MRS studies of human synovial fluid indicate that, as the severity of the disease progresses, arthroscopically graded osteoarthritis is accompanied by increased levels of acetate, glucose/polyols, hydroxybutyrate, and numerous amino acids (17). Acetate is one of the byproducts of polymeric degradation in cartilage and synovial fluid, whereas hydroxybutyrate seems to play a role in regenerating the metabolism of fatty acids in the osteoarthritic joint.

In view of these previous results, the current MRS findings further support the hypothesis that hylan treatment has a salutary effect on the intraarticular environment of the osteoarthritic joint. Our understanding of the mechanisms responsible for the changes in the metabolic profile of osteoarthritic joint fluids observed on MRS is evolving; however, the fact that several of these changes were reversed after late hylan therapy corroborates evidence for the positive effect of viscosupplementation on cartilage metabolism as documented by the gross and histopathological data.

Early (perioperative) hylan treatment did not prevent the development of osteoarthritis or retard the progression of degenerative cartilage changes. These findings are congruent with those of Smith et al. (48), who found no chondroprotective effect when low-molecular-weight hyaluronan therapy was instituted at the induction of osteoarthritis in the unilateral canine model. Accidental or operative anterior cruciate-ligament transection is inevitably associated with hemarthrosis and transient, acute joint inflammation. These data suggest that intraarticular hylan may be ineffectual as a chondroprotective therapy when administered in the face of hemarthrosis and acute joint inflammation.

Given the interanimal variability inherent in experimental canine osteoarthritis, intergroup comparisons are always problematic. However, the severity of osteoarthritis was less marked in the control osteoarthritic knees of the early-treatment group. Thus, early arthrocentesis may also have ameliorated the development and progression of osteoarthritis in this study. This possibility can be best tested by a direct intra-group experiment using the bilateral canine model in which one anterior cruciate ligament-transected knee would undergo early arthrocentesis while the contralateral, anterior cruciate ligament-transected control knee would not.

Three weekly unilateral injections of hylan at the knee joint 2 months after the induction of accelerated bilateral canine osteoarthritis significantly decreased the severity of the disease, as demonstrated by gross and histological indices and corroborated by MRS of synovial fluid. This amelioration of disease progression was documented 6 months after the hylan treatment. Thus, this study provides evidence that three injections of hylan administered relatively early in osteoarthritis can potentially slow subsequent articular cartilage degeneration for at least 6 months.

Intraarticular administration of hylan in the bilateral anterior cruciate-ligament transection and partial neurectomy model of osteoarthritis in dogs improves the gross and histopathological outcome in early osteoarthritis. Thus, in addition to restoring the viscoelastic properties of osteoarthritic synovial fluid, hylan appears to have a direct chondroprotective effect. These results suggest considering hylan therapy in early osteoarthritis. Further work is required to establish the mechanism, or mechanisms, that underlie this chondroprotective effect and to clearly define
the optimal frequency of administration and dosage of hylan to maximize its beneficial effect on articular cartilage.

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