Clearance kinetics of a hylan-based viscosupplement after intra-articular and intravenous administration in animal models

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Abstract: Clearance of hylan fluid and hylan gel, components of the hyaluronan (HA)-derived viscosupplement hylan G-F 20, following intra-articular injection into normal, healthy rabbits was evaluated. Radiolabeled hylan G-F 20 was injected at a volume of 0.3 mL into both knee joints of 12 rabbits. At sacrifice, synovial fluid, joint tissues, blood, popliteal lymph nodes, liver, spleen, kidney, and lung were analyzed for radioactivity. The half-life of the fluid component, a high-molecular weight hylan, was 1.5 ± 0.2 days while the half-life of the hylan gel component, a crosslinked hylan, was 8.8 ± 0.9 days. There was no radioactivity detected in the blood or the major internal organs following intra-articular injection. A rat model was used to evaluate the clearance of a large intravenous bolus of solubilized hylan gel. No accumulation of hylan gel degradation products was observed in any major organs and the half-life of hylan elimination from the blood was within normal ranges for HA elimination. The dosing used in the nonclinical rabbit intra-articular study was equivalent (v/w) to a single 6 mL dose in humans. These results are consistent with the current clinical data that demonstrates safety and effectiveness of an increased volume of hylan G-F 20 injected into the osteoarthritis knee. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 457–462, 2012.

Key Words: hyaluronan, viscosupplementation, hylan G-F 20, osteoarthritis, Synvisc


INTRODUCTION

Hyaluronan (HA)-based intra-articular viscosupplements are a widely used and established treatment for the relief of pain due to osteoarthritis (OA).1 In patients with OA, the synovial fluid is markedly less viscoelastic due to lower concentration and reduced molecular weight of the HA compared with normal synovial fluid.2–4 These reduced viscoelastic properties correlate with pain associated with OA.5 HA-based viscosupplementation aids in the restoration of a normal joint environment;5–7 by augmenting and elevating the viscoelastic properties of the synovial fluid. Studies in equine arthritis established that highly viscoelastic HA preparations provided longer lasting and greater magnitude of pain relief with fewer injections, when compared with HA preparations which were less viscoelastic.7

HA makes up 95% of the glycosaminoglycan content of synovial fluid (based on dry weight).8 The HA component accounts for the viscoelastic properties of synovial fluid and plays a major role in controlling the metabolism in the joint, affecting all fluid flow in the joint through modulation of diffusion and outflow.9–12 The clearance and elimination processes of exogenous HA following intra-articular injection of radiolabeled HA preparations have been studied in rabbits,13 sheep,14 and horses.15 In all three animal models, HA clearance exhibited first-order kinetics with an intra-articular half-life in the range of 12–24 h. Exogenous HA was found to penetrate and coat the synovial tissues and the surface layers of articular cartilage (lamina splendens) and subsequently move through the soft tissues (synovial membrane) and into lymphatic system.16,17 From the lymph, HA flows into the blood circulation and is rapidly cleared and taken up by the liver endothelial cells.18 The systemic elimination of HA has been studied in rabbits and rats and found to be similar to the elimination rate in man.19,20 Following intravenous administration of radiolabeled HA in rabbits and rats, clearance of HA follows first-order kinetics with a half-life of 2–7 min.18–20

Most current HA-based treatment regimens for OA involve multiple (3–5) intra-articular injections of 2–2.5 mL.21 A chemically crosslinked HA product (hylan G-F 20) intended to provide pain relief with a one-injection treatment regimen (Synvisc-One®, Genzyme Biosurgery, Genzyme Corporation, Cambridge, MA) was recently clinically evaluated.22–24 Clinical studies in patients with a diagnosis of knee OA demonstrated that administration of one intra-articular injection of 6 mL of hylan G-F 20 provided significant improvement in pain scores at 6 months post-treatment.22–24 The single 6 mL injection of hylan G-F 20 was well tolerated
in patients, and no increase in the rate of local adverse events compared with saline control was observed.23,25

In a nonclinical study, a rabbit model was used to evaluate the distribution and clearance of radiolabeled hylan G-F 20 components following one intra-articular injection of a volume of 0.3 mL that corresponds to a human regimen of 6 mL. The biosynthetic and in vitro labeling procedures used to incorporate the radiolabels preserved the large polymer structures of the HA components in hylan G-F 20. As export from the intra-articular space is slow, the amount of hylan entering the systemic circulation from the joint is small, and significant levels are not detectable in the circulation using an intra-articular model. Hylan fluid clears more rapidly from the joint than hylan gel and presumably leaves by the same pathway as endogenous HA of synovial fluid. However, the clearance of the hylan gel, a chemically crosslinked HA derivative composed of soft deformable gel particles, clears the joint at a much slower rate and presumably is broken down via mechanical or chemical mechanisms allowing exit from the synovial space into the systemic circulation. Therefore, an intravenous study was conducted in a rat model to evaluate the systemic clearance of the hylan gel (acid solubilized) component of hylan G-F 20. Hylan gel was solubilized and then administered as a large bolus dose via the tail vein in the rat and blood clearance measured. This study permitted detection and quantitative analysis of distribution and elimination of the longer half-life hylan gel component.

MATERIALS AND METHODS

[^14]C-hylan fluid (hylan fluid CAS registry number 507454-10-6) was prepared in vitro through organ culture of freshly harvested chicken combs; the culture medium contained 20 μCi/mL of[^14]C-acetate (ICN Radiochemicals, Irvine, CA) as a metabolic precursor which becomes incorporated into the polysaccharide chain of the hylan fluid during the 72 h incubation (37°C, 5% CO2). Treated combs were frozen, sliced then treated with formaldehyde, and dried according to the method of Balazs et al.26 The formaldehyde treatment forms protein-mediated crosslinks with HA chains resulting in the average molecular weight of the hylan polymer of 4–8 × 10^6 daltons. Radiolabeled hylan was extracted from comb slices using distilled water; and hylan fibers were precipitated from the aqueous extract using 95% ethanol. The radiolabeled hylan fibers were washed with acetone and dried before dissolving in phosphate buffered saline, specific activity 1.87 × 10^6 dpm/mL (1.61 × 10^5 dpm/mg).

[^3]H-hylan gel (hylan gel CAS # 872131-04-09) was produced by the addition of tritiated water ([^3]H2O), 100 mCi/mL, New England Nuclear/DuPont, Boston, MA) during crosslinking, resulting in the covalent attachment of a[^3]H-label to the carbon in the methylene groups of sulfonyl bis-ethyl crosslink and pendant groups (Figure 1).27,28 Loss of[^3]H-label by isotope exchange from the methylene group is minimal at physiological pH.29 Enzymatic degradation of the gel results in cleavage to HA tetramers and hexamers with the pendent group attached and octamers and dodecamers with the crosslink attached,30 indicating that the radioactive label will model the excretion of HA fragments. Equilibrium hydration of the gel was achieved by extensive washing with sterile saline. Specific activity, as determined with a digested aliquot of gel, was 1.58 × 10^6 dpm/mL (371,764 dpm/mg), the polymer content of the gel was 4.25 mg/mL.

Radiolabeled hylan G-F 20

Hylan G-F 20 is composed of 80% (v/v) hylan fluid solution (8–10 mg/mL), molecular weight 4–8 × 10^6 daltons and 20% (v/v) hylan gel (3.5–5.5 mg/mL), a viscoelastic crosslinked HA gel. The radiolabeled test material was prepared in the laboratory as a sterile, nonpyrogenic, and noncytotoxic test material. The radiolabeled hylan fluid was mixed with the radiolabeled hylan gel in the same ratio as the hylan G-F 20 clinical product.

Solubilized[^3]H-hylan gel was prepared from[^3]H-hylan gel that had been prepared according to the procedure described above, then hydrolyzed in 0.15N HCl for 3 h at room temperature and neutralized with 0.1N NaOH. The polymer content of the radiolabeled degraded gel was 4.74 mg/mL; specific activity was 332,627 dpm/mL. The test material was autoclaved before use; the test material was sterile, nonpyrogenic, noncytotoxic, and filterable.

In vivo studies

All animal studies reported here were reviewed and approved by an Institutional Animal Care and Use Committee. Intra-articular (knee joint) administration of radiolabeled ([^3]H/[^{14}]C) hylan G-F 20

Twelve normal, healthy New Zealand White rabbits (pathogen-free, male, Hazelton Research Animals, Denver, PA) weighing between 2.5 and 3.5 kg were used in this study. Rabbits were randomly assigned to one of four groups, three rabbits per group, and anesthetized with 2 mL of a mixture of 54 mg/mL ketamine hydrochloride and 9.2 mg/mL xylazine via intramuscular injection before test material administration. Radiolabeled hylan G-F 20 was administered by intra-articular injection at a volume of 0.3 mL (6 mL/60 kg body weight) per joint. One group of rabbits was sacrificed at each of the following timepoints: 1 day, 3 days, 1 week, and 4 weeks following injection. At each timepoint, synovial fluid, joint tissues, blood, popliteal lymph nodes, liver, spleen, kidney, and lung were collected and analyzed for radioactivity using a dual channel scintillation counter.

Intravenous administration of solubilized[^3]H-hylan gel

Twenty-five normal, healthy female Sprague-Dawley rats (Taconic Farms, Germantown, NY) were randomly assigned to five groups of five rats each. Animals were sedated with ketamine hydrochloride dosed at 22 mg/kg and injected intravenously with a tail vein with ~ 1 mL bolus of solubilized[^3]H-hylan gel that contained 4.74 mg/mL of HA polymer, corresponding to a dose of 22 mg/kg (300 mL/60 kg body weight). At timepoints of 15 min, 1, 3, 6, and 24 h, one group of rats was euthanized and urine (cystocentesis and voided urine), blood (retro-orbital sinus), lungs, liver, spleen, and feces were collected, combusted using a Packard
sample oxidizer, and analyzed for $^3$H using a Packard Model 4430 liquid scintillation spectrometer.

**Statistical analysis**

Data were analyzed using descriptive statistics. The mean plus or minus one standard error of the mean are presented. Half-life calculations were conducted using regression analysis methods.

**RESULTS**

**Intra-articular (knee joint) injection of radiolabeled hylan G-F 20**

Following intra-articular injection of double labeled ($^{14}$C hylan fluid and $^3$H hylan gel) hylan G-F 20, the clearance of the $^{14}$C-hylan fluid component was found to follow first-order kinetics with an apparent half-life of 1.5 ± 0.2 days (Table I and Figure 2). At 7 days post-treatment, 1% of total injected $^{14}$C activity was recovered in the joint tissues. Clearance of the $^3$H-hylan gel component was also found to follow first-order kinetics, with an apparent half-life of 8.8 ± 0.9 days (Table I and Figure 3). At 7 days post-treatment, 20% of total injected $^3$H activity was recovered from the joint tissues.

All measurable radioactivity was found within the joint, with no significant radioactivity detected in the blood or internal organs following intra-articular injection. At 28 days post-treatment, more than 95% of total radioactivity ($^{14}$C and $^3$H) was eliminated from the joint space. Hylan fluid and hylan gel components were found distributed in the joint tissues, with the major amounts at 1 day post-treatment found in the synovial fluid, cartilage surface, synovial membranes, and subpatellar fat pad.

**TABLE I. Intra-articular Clearance ($t_{1/2}$) of Hylan G-F 20 Components following Injection into the Rabbit Knee Joint**

<table>
<thead>
<tr>
<th>Hylan Component</th>
<th>Half Life (Mean ± SEM)</th>
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<tr>
<td>$^{14}$C hylan fluid</td>
<td>1.5 days ± 0.2 days</td>
</tr>
<tr>
<td>$^3$H hylan gel</td>
<td>8.8 days ± 0.9 days</td>
</tr>
</tbody>
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**FIGURE 1.** Chemical structure of radiolabeled hylan gel. The crosslinking reaction of hylan fluid with vinyl sulfone illustrates the incorporation of the tritium ($^3$H) label into the crosslink and pendant moieties.
Intravenous administration of solubilized radiolabeled hylan gel

Elimination of solubilized $^3$H hylan gel from the blood followed first-order kinetics with a half-life of 22 min. Twenty-four hours following intravenous injection of a high dose of $^3$H solubilized hylan gel into rats, total recovery of radioactivity was nearly complete with a mean recovery of radioactivity of 91.6% ± 6.9% (range 74%–102%) from animals in this group. No urine was produced for collection at the 15-min timepoint. Urine at timepoints 1, 3, 6, and 24 h contained on average more than 75% of the total radioactivity injected (Table II).

The average $[^{3}H]$ activity in the blood of each group decreased steadily over time from an average of 10.17% of the total radioactivity at 15 min postinjection to an average of slightly more than 1% of the total radioactivity at 6 h (1.09%) and 24 h (1.17%) (Figure 4). Low percentages of total $[^{3}H]$ activity were recovered from the liver, lungs, and spleen at all timepoints. Very low percentages of total $[^{3}H]$ activity were recovered from the feces; the average percentages were 0.03% at 1 h, 0.0% at 3 h, 0.14% at 6 h, and 7.14% at 24 h postinjection. No feces were produced by the group at the 15-min timepoint. The liver contained the highest percent of injected $[^{3}H]$ activity of all the organs; the $[^{3}H]$ activity recovered in the liver decreased steadily over time from an average of 2.19% at 15 min to an average of 0.59% of the total radioactivity at 24 h postinjection.

**DISCUSSION AND CONCLUSIONS**

The objective of these studies was to evaluate the intra-articular and systemic clearance properties of hylan G-F 20 components using intra-articular and intravenous in vivo models in normal, healthy animals. The clearance of HA from the intra-articular space is through the lymphatic and circulatory systems. The intravenous administration of the hylan gel (acid solubilized) component was used to model the clearance from the blood once the hylan material cleared the knee joint.
Hylan G-F 20 was studied at an intra-articular injection volume corresponding to 6 mL for a human intra-articular injection. The hylan gel component of hylan G-F 20 cleared the joint with a half-life of 8.8 ± 0.9 days while the half-life of hylan fluid was 1.5 ± 0.2 days. The reported half-life of nonmodified HA is ~10 to 13 h. At 4 weeks postinjection, more than 95% of the injected hylan G-F 20 was cleared from the joint. There was no detectable accumulation in the major organs following intra-articular administration.

An intravenous bolus injection of solubilized hylan gel, the longer half-life component, was administered in the rat at a concentration 1000-fold greater than endogenous circulating HA concentration in rat blood. Even at this elevated concentration, the clearance rate of the solubilized hylan gel was consistent with published values for elimination of HA from the rat circulation. The results also demonstrated that when blood levels of hylan are elevated many-fold over those expected following intra-articular administration, elimination rates were maintained, indicating that the systemic capacity for hylan elimination was not saturated. There was little distribution into body tissues, and no accumulation of radiolabeled hylan degradation products was observed in any major organs. In addition, there was near total recovery of injected radioactivity at 24 h.

Clinically, administration of a single injection of 6 mL of hylan G-F 20 or one treatment regimen of three weekly injections of 2 mL of hylan G-F 20 provides therapeutic benefit (relief from pain in the knee due to OA) for up to 26 weeks. The safety of hylan G-F 20 when administered as a single 2 mL intra-articular injections (one course of treatment) to patients with OA has been established in prospective, randomized clinical trials. Clinical studies of a single 6 mL injection of hylan G-F 20 found the frequency and type of adverse events were similar between the group of patients that received hylan G-F 20 and the group that received saline control.

The results from the intra-articular clearance rate study indicate an increase in the half-life of hylan G-F 20 components in the joint as compared with published half-life values of 10 to 13 h for noncrosslinked HA. This longer residence time may provide an extended therapeutic benefit.

In conclusion, clearance of hylan G-F 20 after intra-articular injection in the normal, healthy rabbit exhibits elimination kinetics and distribution properties similar to those reported for HA. The intra-articular half-life of hylan G-F 20 components, though prolonged as compared with HA, is not associated with detectable disruption or alteration of normal joint metabolism. As a result of the extended half-life of hylan gel, hylan is still present within the joint for more than 3 weeks post intra-articular injection. Intravenous administration of a large bolus dose of the solubilized gel component in rats exhibited elimination kinetics and distribution properties similar to those reported for HA and suggest there is no disruption or alteration of normal systemic HA metabolism. These nonclinical study results support the use of a single 6 mL injection in humans and are consistent with the current clinical data which demonstrate safety and effectiveness of an increased volume of hylan G-F 20 injected into the OA knee.

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