Effects of an orally administered mixture of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate on synovial fluid chondroitin sulfate 3B3 and 7D4 epitope in a canine cruciate ligament transection model of osteoarthritis

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Introduction

Of recent interest has been the treatment of osteoarthritis (OA) with compounds such as chondroitin sulfate and glucosamine.1–5 These oral products have been classified as symptomatic, slow-acting disease-modifying OA agents.3,6 In some European countries chondroitin sulfate and glucosamine sulfate are registered as prescription drugs, whereas in North America they are considered as nutraceuticals and food supplements. Glucosamine is an amino monosaccharide used in the synthesis of disaccharide units of glycosaminoglycan (GAG).5 Both the large aggregating proteoglycan (aggrecan) and the small, non-aggregating proteoglycans (biglycan, decorin and fibromodulin) of articular cartilage are composed largely of GAG chains. Keratan sulfate, and chondroitin 4- and 6-sulfates are the main GAGs of articular cartilage.7 Also manganese is a trace element and cofactor in the biosynthesis of GAG.3 Ascorbate is required for collagen fibril formation and cross-linking, but it is non-essential to dogs because of their capacity for endogenous hepatic synthesis of ascorbate.

The rationale for using nutraceuticals is that provision of precursors of cartilage matrix in excess quantities may favor matrix synthesis and repair of articular cartilage. A mixture containing chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate was chondroprotective in a rabbit knee instability model.8 In addition to potential biosynthetic effects, glucosamine may have cyclooxygenase-independent, anti-inflammatory properties.9,10 Also, IL-1 or retinoic acid-induced cleavage of aggrecan by aggrecanase in vitro was inhibited by glucosamine.11 Although there are several reports describing clinical trials of oral chondroitin sulfate alone, or in combination with glucosamine, that document symptomatic improvement in humans and animals with OA, little is known about the mechanisms of action of these compounds.2,4,6,12,13 Early in the process of OA there is increased synthesis and turnover of matrix proteoglycans and collagens by chondrocytes.14–19 One feature of this altered matrix synthesis is that some of the newly formed chondroitin sulfates...
Treatment groups (chondroitin sulfate-glucosamine hydrochloride-manganese ascorbate and cranial cruciate ligament reconstruction) of dogs with unilateral osteoarthritis induced by cranial cruciate ligament transection at time zero

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>Sham arthotomy at 4 weeks</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>Daily oral CS–G–M commencing day one and sham arthotomy at 4 weeks</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>Arthrotomy and CCL reconstruction at 4 weeks</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>Daily oral CS–G–M commencing day one, and arthrotomy and CCL reconstruction at 4 weeks</td>
</tr>
</tbody>
</table>

CS–G–M = chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate.
R–CCL = cranial cruciate ligament reconstruction.

have an increased chain length, and altered sulfation patterns and structure, exposing unique epitopes that are detectable by immuno-assay using monoclonal antibodies such as 3B3 and 7D4.20–23 These antibodies have been used to demonstrate modulation of chondroitin sulfate structure in both articular cartilage and synovial fluid in OA secondary to knee injury in man and animal models.24–26 They are also potentially useful in monitoring disease progression and response to therapy.27

The canine cranial cruciate ligament (CCL—equivalent to anterior cruciate ligament in man) transection model of induced OA was used in our study because it is well characterized and clinically relevant, and it permits longitudinal studies of synovial fluid composition.14–19,28 However, a shortcoming of this model is that chronic joint instability contributes to ongoing deterioration in the biochemical and mechanical properties of articular cartilage, so potentially some subtle benefits of oral chondromodulating or chondroprotective agents may be obscured or overwhelmed.29 Thus we felt that it was important to examine also the effects of these agents in CCL transected joints that had been reconstructed. Therefore, the purpose of our study was to use a canine model of OA induced by CCL transection to examine the effects of orally-administered chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate, both with and without CCL reconstruction. We hypothesized that these orally administered compounds would modulate cartilage matrix synthesis in joints with OA, and that this effect would be reflected by alterations in levels of chondroitin sulfate 3B3 and 7D4 epitope and GAG in synovial fluid.

**Materials and methods**

**ANIMALS AND OSTEOARTHRITIS MODEL**

Sixteen adult pure bred hounds of either sex, weighing 23–32 kg and with no clinical or radiographic evidence of joint disease were used for the study, which was conducted according to AALAC guidelines. The right or left hind limb was randomly selected and the CCL was surgically transected through a medial arthrotomy in all dogs.30 The contralateral limb served as a non-operated, negative control. Dogs were randomly assigned to one of four experimental groups (Table I).

**CHONDROITIN SULFATE, GLUCOSAMINE HYDROCHLORIDE AND MANGANESE ASCORBATE ADMINISTRATION**

Gelatin capsules, each containing a commercial formulation of 200 mg low molecular weight chondroitin sulfate (95% sodium chondroitin sulfate and 5% mixed GAG; TRH122®), 250 mg glucosamine hydrochloride (FCHG49®) and 5 mg manganese ascorbate (CS–G–M; Cosequin®; Nutramax Laboratories®, Inc, Edgewood, MD) were used for the study. The sodium chondroitin sulfate was derived from bovine trachea and the glucosamine was from chitin of crustacean shells. Commencing one day after CCL transection, each dog in Groups II and IV (Table I) was dosed orally with three capsules every 12 hours, for 30 days. Thereafter, each of these dogs was given two capsules every 12 hours until the time of euthanasia at 5 months.

**CRANIAL CRUCIATE LIGAMENT RECONSTRUCTION**

Four weeks after CCL transection, six dogs (Groups I and II) underwent a sham arthotomy on the CCL deficient stifle, to serve as a non-reconstructed control. Ten dogs (Groups III and IV) had CCL reconstruction by isometric placement of an intracapsular graft, as previously described.31 Briefly, an intracapsular graft was fashioned from distal fascia lata and patella tendon. Distally, the graft and its insertion onto the tibial plateau were freed with an osteotome. A tibial tunnel was created to enter the joint at the most caudal point of insertion of the normal CCL. A generous notchplasty was made to protect the graft and permit adequate visualization of the caudo-proximal point for entry of a femoral tunnel into the joint. The femoral tunnel was made and the graft placed through each tunnel. The limb was positioned at an angle of 135° and approximately 1.4 kg of tension was placed on the graft that was then sutured to the lateral femoro-fabellar ligament.

**POST-SURGICAL MANAGEMENT**

For analgesia, all dogs were given buprenorphine (0.03 mg/kg IM) immediately prior to surgery and again during recovery, and the limb was maintained in a soft padded bandage for 3–4 days. Dogs were housed in 2.5 × 1.2 m runs and exercised by leash walking for 15 minutes every third day until the time of euthanasia and necropsy 5 months post-operatively. Morphologic data on joints were reported previously.32

**SYNOVIAL FLUID**

Synovial fluid was collected from left and right stifle joints by direct arthrocentesis at time zero and 1 month while dogs were anaesthetized for surgery. Further synovial fluid samples were collected at 3 months while dogs were sedated, and at 5 months immediately after euthanasia. Synovial fluid was centrifuged at 12000 g for 30 min at 4°C,
and the supernatant stored at —80°C until assayed. Labeling of tubes was in code and investigators performing synovial fluid assays were blinded to treatment groups.

**SULFATED GLYCOSAMINOGLYCAN CONTENT OF SYNOVIAL FLUID**

Aliquots of synovial fluid supernatant were digested in buffered papain solution (20 units/mg protein, Sigma Chemical Co., St Louis, MO, U.S.A.) as described previously. After inactivation of the enzyme by addition of iodoacetic acid, supernatants were treated with hyaluronidase *(streptomyces hyalurolyticus, 2000 TRU/mg; Seikagaka Co., Japan)*, and then sulfated GAG levels were measured using a dye-binding assay. Shank chondroitin sulfate standards (Sigma Chemical Co., St Louis, MO, U.S.A.) in the range of 0–40 µg/ml were prepared. After addition of 1.9 dimethylmethylene blue (Molecular Probes, Inc., U.S.A.) solution to each plate well (Immulon 2, Dynatech Lab, Inc., U.S.A.) containing sample or standard, shifts in absorbency were detected immediately at 530 nm on a plate reader (EL 312e Bio-Kinetics Microplate Reader, Bio-Tek Instruments, VT, U.S.A.).

**3B3 ASSAY**

A competitive equilibrium ELISA based on a previously described method was used with the following variations to quantify 3B3 epitope levels in synovial fluid. Synovial fluid samples were diluted 1:10 in saline. Standards of porcine aggrecan core protein (a generous gift from Professor Michael T. Bayliss, London) in the range 1.95–500 ng/ml were prepared, and monoclonal antibody 3B3 (Seikagaka Co., Tokyo, Japan) at dilution of 1:1600 (final dilution 1:3200) was used. Blank (Tris buffer only) and reference wells (antibody only, no competing antigen) were used for the calibration. 3B3 levels were measured using a curve of standard 3B3 levels from synovial fluid samples diluted 1:10 in saline. The 3B3 levels were calculated in equivalent weight of the antigen standard, using the absorbancy compared to reference wells (no antigen), and by reading this value against the standard curve of percentage of inhibition against the standard antigen concentration.

**7D4 ASSAY**

This assay was similar to the 3B3 ELISA, with the following exceptions. Monoclonal antibody 7D4 was a generous gift of Professor Bruce Caterson (University of Cardiff). Antigen used for coating microplates and preparation of standards was bovine laryngeal cartilage proteoglycan generously provided by Professor Michael T. Bayliss. Plates were coated at 1 µg/ml and standards ranged from 0.0156 to 4 µg/ml of sulfated GAG, as determined by the GAG dye-binding assay. Synovial fluid samples were diluted 1:20 in saline. The 7D4 was applied at a dilution of 1:25 000 (final dilution 1:50 000). Second antibody IgM was diluted 1:1000. Plates were read at 2 h after addition of peroxidase substrate on the plate reader.

**DATA ANALYSIS**

Due to heterogeneity in variance of the data obtained from synovial fluid analyses, raw values were log (natural) transformed prior to performing statistical analyses on 3B3, 7D4 and GAG data, but not the 3B3/GAG or 7D4/GAG ratios. Where appropriate, data have been presented in our results after back transformation to geometric means. Cross-sectional analyses were performed to compare mean synovial fluid levels of 3B3, 7D4, GAG, 3B3/GAG and 7D4/GAG between CCL-transected knees. Also, cross-sectional analyses were performed to compare ratios of synovial fluid levels of 3B3, 7D4 and GAG in CCL-transected knee over contralateral nonoperated knee. A cross-sectional analysis of 1 month data compared the CS–G–M groups (II and IV) to the non-CS–G–M groups; the latter group had a lower mean value. We calculated that 27 dogs per group...
Cross-sectional analysis of effect of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate, and cranial cruciate ligament reconstruction on synovial fluid 1 month after cranial cruciate ligament transection: ratio of operated knee to contralateral non-operated knee*

<table>
<thead>
<tr>
<th></th>
<th>CS–G–M† (N=8)</th>
<th>Non-CS–G–M† (N=8)</th>
<th>P-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>3B3</td>
<td>0.998</td>
<td>0.940</td>
<td>0.163</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>(0.946, 1.050)</td>
<td>(0.864, 1.016)</td>
<td></td>
</tr>
<tr>
<td>7D4§</td>
<td>1.637</td>
<td>1.487</td>
<td>0.367</td>
</tr>
<tr>
<td>(ug/ml)</td>
<td>(1.348, 1.926)</td>
<td>(1.242, 1.732)</td>
<td></td>
</tr>
<tr>
<td>GAG†</td>
<td>1.289</td>
<td>1.289</td>
<td>0.999</td>
</tr>
<tr>
<td>(ug/ml)</td>
<td>(1.177, 1.400)</td>
<td>(1.108, 1.469)</td>
<td></td>
</tr>
</tbody>
</table>

*Figures are arithmetic means and 95% confidence intervals within groups.
†Chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate.
‡Resulting from a two sample t-test.
§Data available for only seven dogs in non-CS–G–M group (15 dogs in total).
¶Data available for only five dogs in non-CS–G–M group (13 dogs in total).

Results of the 1 month cross-sectional analyses comparing mean ratios of values for CCL-transected to contralateral non-operated knees are presented in Table III. There were no significant differences in 3B3, 7D4 and GAG levels between the CS–G–M and non-CS–G–M groups. The 95% confidence intervals for the 3B3 data included unity and therefore differences between operated and non-operated knees were not significant. However, means and 95% confidence intervals for 7D4 and GAG data were above unity, indicating significantly higher 7D4 and GAG levels in synovial fluids of CCL-transected knees compared to non-operated knees, irrespective of CS–G–M treatment.

LONGITUDINAL ANALYSIS

Results of analyses comparing mean synovial fluid levels from the CCL-transected knees (Fig. 1), and also mean ratios of synovial fluid levels in CCL-transected knee over contralateral non-operated knee are presented in Table VI. There was a significant overall difference in 7D4 and 7D4/GAG levels between CS–G–M and non-CS–G–M groups (P=0.012); the latter group had a lower mean value. There were no significant effects with respect to CCL reconstruction. There was a significant time effect for all 3B3, 7D4 and GAG absolute values and ratios, indicating that CCL transection resulted in a significant change in these variables compared to time zero, and also compared to the contralateral non-operated knee. As there were no significant time×CS–G–M or time×CCL reconstruction interactions there was not any evidence of a difference in time effect between groups (Table VI).

Discussion

Clinical trials have suggested that nutraceuticals or slow-acting disease-modifying OA agents contribute to symptomatic relief of pain in humans and animals with osteoarthritis and cartilage degeneration.

Table IV

Cross-sectional analysis of effect of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate, and cranial cruciate ligament reconstruction on synovial fluid in operated knee joints 3 months after cranial cruciate ligament transection

<table>
<thead>
<tr>
<th></th>
<th>CS–G–M (N=8)</th>
<th>Non CS–G–M (N=8)</th>
<th>P-value*</th>
<th>Reconstruction (N=10)</th>
<th>No reconstruction (N=6)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3B3‡§</td>
<td>248.4</td>
<td>181.1</td>
<td>0.029</td>
<td>204.9</td>
<td>229.5</td>
<td>0.412</td>
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<tr>
<td>(ng/ml)</td>
<td>(207.5, 297.5)</td>
<td>(138.6, 236.5)</td>
<td></td>
<td>(157.8, 266.2)</td>
<td>(183.2, 287.4)</td>
<td></td>
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<tr>
<td>7D4§</td>
<td>71.5</td>
<td>47.8</td>
<td>0.036</td>
<td>52.1</td>
<td>70.9</td>
<td>0.108</td>
</tr>
<tr>
<td>(ug/ml)</td>
<td>(48.7, 105.0)</td>
<td>(38.8, 59.0)</td>
<td></td>
<td>(42.3, 64.3)</td>
<td>(40.6, 70.9)</td>
<td></td>
</tr>
<tr>
<td>GAG†</td>
<td>53.9</td>
<td>54.3</td>
<td>0.998</td>
<td>50.6</td>
<td>59.8</td>
<td>0.758</td>
</tr>
<tr>
<td>(ug/ml)</td>
<td>(35.8, 81.7)</td>
<td>(41.1, 71.7)</td>
<td></td>
<td>(38.3, 66.7)</td>
<td>(36.4, 98.5)</td>
<td></td>
</tr>
<tr>
<td>3B3/GAG‡¶</td>
<td>6.26</td>
<td>3.57</td>
<td>0.202</td>
<td>4.63</td>
<td>4.23</td>
<td></td>
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<tr>
<td>(ng/ug)</td>
<td>(2.83, 7.69)</td>
<td>(2.36, 4.78)</td>
<td></td>
<td>(2.54, 6.72)</td>
<td>(2.13, 6.34)</td>
<td></td>
</tr>
<tr>
<td>7D4/GAG¶</td>
<td>1.34</td>
<td>0.92</td>
<td>0.007</td>
<td>1.10</td>
<td>1.21</td>
<td>0.425</td>
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<tr>
<td>(ug/ug)</td>
<td>(1.21, 1.46)</td>
<td>(0.62, 1.23)</td>
<td></td>
<td>(0.82, 1.38)</td>
<td>(0.95, 1.47)</td>
<td></td>
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</table>

*Resulting from a two-way ANOVA: testing for CS–G–M effect.
†Resulting from a two-way ANOVA: testing for CCL reconstruction effect.
‡Data available for only nine dogs in reconstruction group, seven in non-CS–G–M group (15 dogs in total).
§Figures are geometric means and 95% confidence intervals within groups.
¶Figures are arithmetic means and 95% confidence intervals within groups.
Cross-sectional analysis of effect of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate, and cranial cruciate ligament transection on synovial fluid 3 months after cranial cruciate ligament transection: ratio of operated to contralateral non-operated knee

Figure 1. Synovial fluid concentrations of 3B3 (A) and 7D4 (B) in cranial cruciate ligament transected knees of dogs given an oral mixture of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate (CS–G–M). (Data shown are geometric means for dogs with complete data sets at all four time points.) *Differences between groups in 3B3 and 7D4, by cross-sectional analysis at 3 months are significant (P<0.05). †Differences between groups by longitudinal-analysis over five months are significant (P=0.012).

Table V

<table>
<thead>
<tr>
<th></th>
<th>CS–G–M (N=8)</th>
<th>Non-CS–G–M (N=8)</th>
<th>P-value†</th>
<th>Reconstruction (N=10)</th>
<th>No reconstruction (N=6)</th>
<th>P-value‡</th>
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<tbody>
<tr>
<td>3B3§ (ng/ml)</td>
<td>1.12</td>
<td>1.09</td>
<td></td>
<td>1.07</td>
<td>1.16</td>
<td>0.165</td>
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<tr>
<td>(N=6)</td>
<td>(1.03, 1.23)</td>
<td>(0.97, 1.20)</td>
<td>0.397</td>
<td>(1.00, 1.15)</td>
<td>(1.02, 1.30)</td>
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<tr>
<td>7D4 (ug/ml)</td>
<td>1.52</td>
<td>1.53</td>
<td></td>
<td>1.48</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.36, 1.69)</td>
<td>(1.35, 1.71)</td>
<td>0.935</td>
<td>(1.35, 1.62)</td>
<td>(1.37, 1.79)</td>
<td>0.360</td>
</tr>
<tr>
<td>GAG § (ug/ml)</td>
<td>1.19</td>
<td>1.10</td>
<td></td>
<td>1.13</td>
<td>1.19</td>
<td>0.370</td>
</tr>
<tr>
<td>(N=6)</td>
<td>(1.08, 1.30)</td>
<td>(1.02, 1.18)</td>
<td>0.097</td>
<td>(1.09, 1.17)</td>
<td>(1.02, 1.36)</td>
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</tbody>
</table>

*Figures are arithmetic means and 95% confidence intervals within groups.
†Resulting from a two-way ANOVA allowing for CS–G–M treatment.
‡Resulting from a two-way ANOVA allowing for CCL reconstruction.
§Data available for only nine dogs in CCL reconstruction group (12 in total).
∥Data available for only eight dogs in CCL reconstruction group (14 in total).
¶Data available for only six dogs in CCL reconstruction group (12 in total).

OA,2,4,12,13,36 but the mechanisms by which these compounds act are largely unknown. Evidence derived from in vitro studies suggest that alterations in proteoglycan biosynthesis, as well as anti-inflammatory, anti-catabolic and chondroprotective actions may contribute to the beneficial effects observed clinically.5,8,10,11,37,38 Our study of a canine model of surgically induced OA is among the first to provide in vivo evidence that chronic oral administration of CS–G–M resulted in modulation of articular cartilage metabolism that was reflected in synovial fluid 3B3 and 7D4 epitope concentrations. These findings are in accord with morphologic observations that Mankin scores and joint capsule thickening were less in CS–G–M treated dogs.32 The administration of CS–G–M over the course of 5 months resulted in significant alterations in 7D4 and 7D4/GAG concentrations in synovial fluid of CCL transected knees. Also, in cross-sectional analysis at three months, levels of 3B3, 7D4 and 7D4/GAG ratio were significantly elevated in the CCL-transected knees in CS–G–M treated dogs, compared to non-CS–G–M treated dogs. However, when synovial fluid variables in the CCL-transected knees were expressed as a ratio to the contralateral nonoperated knee, there were no significant treatment effects due to CS–G–M, suggesting that the effects of these compounds on synovial fluid composition were systemic and not localized to osteoarthritic joints. Pharmacokinetic studies in dogs and other animals have shown that both glucosamine and chondroitin sulfate are absorbed from the gastrointestinal tract after oral administration,39,40 and radiolabeled chondroitin sulfate was incorporated into normal articular cartilage.7 Our results suggest that the effects of these compounds in vivo were to modulate GAG structure in both normal and osteoarthritic joints, such that an absolute increase in 3B3 and 7D4 epitope antigenic sites resulted. Their effects were not simply due to increased GAG synthesis by chondrocytes as had been suggested previously,26 because there were no significant differences between groups (CS–G–M vs non-CS–G–M) in total GAG content of synovial fluid.

It has been proposed that 3B3 and 7D4 are ‘anabolic’ markers of cartilage turnover, and result from attempts by chondrocytes to repair or remodel damaged cartilage in OA, but they are not definitive ‘markers’ of OA.27 Monoclonal antibody 3B3 recognizes an epitope on chondroitin sulfate chains that have a non-reducing termination of GlcAlf,3GalNAc6S.41 The proportion of such terminal disaccharides, at about 9%, remains constant throughout
life but the immunoreactivity of the proteoglycans varies with stage of development, maturity and pathology of connective tissues, because chain length and presentation are critical factors for recognition of chain terminations by monoclonal antibody 3B3.\textsuperscript{31} Proteoglycans isolated from osteoarthritic cartilage and synovial fluid showed increased immunoreactivity to 3B3,\textsuperscript{24,27,42} which may in part have been due to alterations in the sulfation and length of chains ending with the disaccharide containing the 3B3 epitope.\textsuperscript{43} The epitope for monoclonal antibody 7D4 is less well characterized, but 7D4 appears to recognize subtle combinations of sulfated and non-sulfated disaccharide isomers within the native chondroitin sulfate chain.\textsuperscript{27} Expression of the 7D4 epitope is also more prevalent in proteoglycans extracted from osteoarthritic cartilage and synovial fluid.\textsuperscript{27,44}

In our model, transection of the CCL for the purpose of induction of secondary OA was responsible for much greater alterations in synovial fluid levels of 3B3, 7D4 and GAG than CS–G–M, both over time and as compared to the contralateral non-operated knee. The one exception occurred at 1 month after CCL transection, when 3B3 in the operated knee was not significantly different from the contralateral non-operated knee, but by 3 months levels of 3B3 in the CCL-transected knee were significantly greater. This suggests that there is a lag period of about 4 weeks after induction of the process of OA in this model before GAG containing the 3B3 epitope begins to be released from articular cartilage into synovial fluid in increased quantities, which perhaps relates to delayed upregulation of aggrecan mRNA expression in articular cartilage.\textsuperscript{45} This delay was not detected in a previous study of the same model in which synovial fluid 3B3 levels were only measured at 6 and 12 weeks after CCL transection.\textsuperscript{46} Another important difference between our study and the previous one\textsuperscript{46} was that we collected synovial fluid by direct aspiration arthrocentesis and performed ELISA on known dilutions of synovial fluid, whereas others have used saline lavage of joints to facilitate collection of synovial fluid,\textsuperscript{46} so final concentration of epitope in synovial fluid could not be accurately determined. We have observed that saline dilution of synovial fluid causes non-linear and unpredictable increases in measured levels of 3B3 epitope by ELISA, perhaps because there is unmasking or increased presentation of antigenic sites (Johnson KA, unpublished data).

By contrast to the observed changes in 3B3, we found that CCL transection induced a significant elevation in both 7D4 and 7D4/GAG at 1 month that was sustained over the following 4 months. Total GAG content of synovial fluid in CCL-transected joints also increased over time, and this change corresponds with the findings of other studies of canine knees with OA secondary to naturally occurring CCL rupture in which GAG content was elevated in early stages, but in chronic disease declined and was negatively correlated to radiographic scores.\textsuperscript{47,48} This is also consistent with findings in chronic knee OA in humans.\textsuperscript{49,50} Cranial cruciate ligament reconstruction had no significant effect on synovial fluid variables, suggesting that attempts to eliminate anterior drawer motion in the knee with an anatomical autologous reconstruction has no significant effect on the alterations in articular cartilage metabolism that are initiated by surgically induced joint instability. This is consistent with the observations that reconstruction of the anterior cruciate ligament in humans does not prevent the late development of secondary OA, in spite of the improvement in function and joint stability.\textsuperscript{51,52}

It is important to appreciate that our study focused on changes in synovial fluid, as reflections of structural changes in articular cartilage. Advantages of longitudinal studies of synovial fluid markers over magnetic resonance imaging or arthroscopy are economics and non-invasiveness. However, it is assumed that most GAG in synovial fluid is derived from the articular cartilage and not the meniscal cartilages or synovial membrane. The present study did not evaluate lameness or joint function and further study is needed to elucidate mechanisms by which CS–G–M produces symptomatic alleviation of pain in OA. However, our study has provided evidence from an in vivo model of OA that chronic oral administration of CS–G–M resulted in altered synovial fluid 3B3 and 7D4 epitope concentrations which may be a reflection of a modulation in articular cartilage metabolism. Until our understanding of the significance of these epitopes is more complete, it may be premature to try to say whether the observed effects of CS–G–M were beneficial to osteoarthritic articular cartilage.

### Acknowledgments

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References


