OsteoArthritis and Cartilage (2004) **12,** 232–238

doi:10.1016/j.joca.2003.11.007

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Intra-articular hyaluronate in experimental rabbit osteoarthritis can prevent changes in cartilage proteoglycan content[1]

D. J. S. Hulmes Ph.D†,#*, M. E. Marsden†, R. K. Strachan F.R.C.S.Ed.‡, R. E. Harvey Ph.D¶, N. McInnes Ph.D.¶ and D. L. Gardner M.D.§[2]

† *Department of Biochemistry, University of Edinburgh, Edinburgh, EH8 9XD UK*
‡ *Department of Orthopaedic Surgery, University of Edinburgh, Edinburgh, EH8 9XD UK*
§ *Department of Pathology, University of Edinburgh, Edinburgh, EH8 9XD UK*
¶ *Fermentech Medical Ltd., Heriot-Watt Research Park, Riccarton Campus, Edinburgh EH14 4AS, UK*
# *Institut de Biologie et Chimie des Protéines, 7 passage du Vercors, 69367 Lyon cedex 7, France*

Summary

Objective: The purpose of this study was to determine the effects of intra-articular injections of high molecular weight (2000 kDa) sodium hyaluronate (HA) on the progression of articular cartilage degeneration in a rabbit partial medial meniscectomy model of osteoarthritis.

Design: Six experimental groups included normal, sham operated, and operated and injected animals, the latter injected once-weekly (for two weeks or twelve weeks, beginning four weeks after surgery) with either 1% (w/v) HA or phosphate buffered saline (PBS). Following assessment of gross morphology, serial adjacent blocks of full-depth articular cartilage were prepared from the tibial condyle for analysis of total water, hydroxyproline, DNA and proteoglycan (uronic acid) content, as well as the ratio of galactosamine to glucosamine. Samples were sub-divided into inner (medial) and outer (lateral) regions.

Results: No morphological differences were recognized between joints injected with PBS and those receiving HA. When analysed biochemically, there were no significant differences in hydration, hydroxyproline or DNA content between the experimental groups. In contrast, HA injection did affect changes in proteoglycan content. Expressed per tissue dry weight, uronic acid content in the operated group injected with PBS for two weeks was lower than normal ($P<0.02$), a result not seen in the corresponding HA injected group. After 12 weeks of PBS injections, uronic acid content (per dry weight) was higher than normal ($P<0.01$), an effect again not observed in the corresponding HA injected group. Results for the galactosamine: glucosamine ratio showed a reduction after 12 weeks of injections, but no differences between PBS and HA injected groups.

Conclusions: Once-weekly, intra-articular injection of high molecular weight HA can prevent changes in proteoglycan content in tibial condylar articular cartilage, compared to PBS injected controls, in the rabbit partial meniscectomy model of osteoarthritis.

Key words: Articular cartilage, Hyaluronate, Osteoarthritis, Proteoglycans, Rabbit.

Introduction

Osteoarthritis (OA) is characterized by the progressive degeneration of articular cartilage, with an accompanying re-modeling of both cartilage and bone[1,2]. An early event in the pathogenesis of OA is a loss of hyaluronate (HA) from the synovial fluid, together with a decrease in its average molecular weight[3]. These changes in HA result in a marked diminution in the rheological properties of the synovial fluid, which may lead to degeneration or re-modeling of the articular cartilage and underlying bone. In order to prevent these degenerative changes, the concept of visco-supplementation was introduced by Balazs and colleagues[3], in which exogenous hyaluronate is injected intraarticularly into the joint. Though intra-articular injection with HA is becoming increasingly widespread in the treatment of knee OA[4,5], its efficacy remain controversial[6–11]. Recommendations differ in the number and frequency of injections required to bring about significant improvement, and the molecular weight (MW) of the HA used is clearly an important factor[12].

Many animal models of osteoarthritis have been developed. These include transection of the anterior cruciate ligament in dogs[13–18], rabbits[19] and rats[20]. Further rabbit models include joint immobilization[21,22] or partial meniscectomy[23–25], sometimes combined with ligament transection[26]. Total meniscectomy models have also been used in rabbits[27,28] and sheep[29,30]. Here we report the effects of intraarticular, high MW (2000 kDa) HA injections

[1] Supported by the Department of Trade and Industry (UK), the Scottish Home and Health Department, the Wellcome Trust and the University of Edinburgh.

*Address correspondence to: Dr D. J. S. Hulmes, Institut de Biologie et Chimie des Protéines, CNRS UMR 5086 7, passage du Vercors, 69367 Lyon Cedex 07, FRANCE. Tel.: +33-4-72722667; Fax: +33-4-72722604; E-mail: d.hulmes@ibcp.fr

[2] Present addresses: (MEM) Respiratory Medicine Section, Dept of Medical and Radiological Services, University of Edinburgh, Edinburgh EH8 9AG, UK; (RKS) Dept Orthopaedic Surgery, Charing Cross Hospital, Fulham Place Rd, London W6 8RF, UK; (REH) DePuy International Ltd, St Anthony's Road, Leeds, N Yorks LS11 8DT, UK; (NM) Danisco Beaminster Ltd, North St, Beaminster, Dorset DT8 3DZ, UK.

Received 27 March 2003; revision accepted 2 November 2003.

on biochemical composition of rabbit articular cartilage in a partial meniscectomy model of OA[24].

Materials and methods

Eighty-four mature New Zealand white rabbits of mean weight 3.0 kg were obtained from an accredited supplier and maintained on a balanced diet under Home Office-approved conditions. Seventy-eight of these animals were selected for the present study.

SURGICAL PROCEDURE

Pilot studies showed that a modified Hulth[31] procedure caused cartilage disease of such severity that sensitive histological and cytological analyses were not practicable. To promote milder disease, the right knee joints of 60 rabbits were therefore subjected only to partial meniscectomy. The anteromedial capsule of the right knee was opened and the medial meniscus identified. Release of the medial collateral ligament gave access to the posterior part of the capsule. All capsular attachments of the medial meniscus were freed. The meniscus was retracted from the joint and the posterior meniscotibial ligament divided. The greater part of the meniscus was removed from the joint. Care was taken at all times to avoid contact between the instruments and articular surfaces. The joint capsule was repaired, the skin closed and a well-padded dressing applied. The joint was left stable to both valgus and varus movement and to anteroposterior stressing. The animals were maintained in large cages for the first 24 h postoperatively. In a sham operation, a surgical incision was made into the knee joints of a further 12 animals without excising a meniscus. Six animals were retained as normal controls.

HA INJECTIONS

Further pilot studies demonstrated that a period of at least 3 weeks was necessary before the knee joints of operated animals could be injected safely with viscous HA. Without this precaution, the polymer was found to escape from the joint into adjacent soft tissues, predisposing to infection. Thirty operated animals were therefore injected once weekly intra-articularly with sterile, 0.5 ml endotoxin-free, 1% w/v sodium hyaluronate solution (Fermentech Medical Ltd), molecular mass 2×10^6 Da (obtained by bacterial fermentation of *Streptococcus equi*), in phosphate buffered saline (PBS), pH 7.2, beginning 4 weeks after surgery. Injections were given for 2 weeks to 15 rabbits and for 12 weeks to the remaining 15. Thirty further animals were injected in the same way once weekly with 0.5 ml sterile PBS, pH 7.20, 15 for 2 weeks and 15 for 12 weeks.

TISSUE COLLECTION

Both right and left knee joints were studied in the normal control group (6 rabbits). The right knee joints of the sham-operated group (12 rabbits) were analysed at the end of the 4 week recovery period.

Animals were sacrificed by intravenous injection of pentobarbitone. The lower limbs were excised and placed on ice. The right knee joints were opened through a lateral incision, the cruciate and collateral ligaments divided and each femur separated from the corresponding tibia. After removal of the lateral meniscus and the remaining part of the medial meniscus, the tibial condylar cartilages were excised superficial to the calcified cartilage zone. With a dissecting microscope and ophthalmic scalpel, a circumferential incision encircled the lateral and medial aspects of the tibial condyle. Nine to 11 perpendicular incisions were made in the coronal plane across each condylar cartilage, commencing posteriorly. The blocks thus defined were undercut, allowing ~7.0×0.9×0.5 mm samples to be removed. Blocks were pooled and immediately placed in pre-weighed, sealed containers that were previously humidified with moist tissue paper.

BIOCHEMICAL PROCEDURES

Coronal blocks of articular cartilage, selected as above, were bisected sagitally in a humidified environment. The first sample from each specimen therefore represented the relatively thick, inner (medial) region of the tibial condylar cartilage normally covered by a relatively thin part of the meniscus whereas the second sample represented the thin, outer (lateral) region normally covered by a relatively thick part of the meniscus. Each sample was then weighed quickly before being dried, in a 60°C oven for 4 h, to constant weight. Dried cartilage samples (3–25 mg dry weight) were solubilized by sodium dodecyl sulphate and proteinase K digestion, as described[32]. Following digestion, DNA, uronic acid, hydroxyproline content, as well as galactosamine/glucosamine ratios, were determined as below.

Hexuronic acid was determined by a modification of the carbazole method of Bitter and Muir[33,34]. To prevent caramelization of carbohydrate, the borate/sulphuric acid solution was cooled to –30°C before the addition of sample or glucurolactone standard. Total DNA content was determined by the Hoechst 33258 fluorescent dye-binding technique[32]. For hydroxyproline analysis, digests were hydrolysed in 6 M HCl in sealed tubes for 16 h at 100°C. After removal of HCl by evaporation in a SpeedVac concentrator, hydroxyproline was determined colorimetrically after chloramine T oxidation and subsequent reaction with p-dimethylaminobenzaldehyde[35]. Galactosamine/glucosamine ratios were determined[36] following hydrolysis of cartilage digests in 2M trifluoroacetic acid (TFA) for 4 h at 100°C. The TFA was removed by evaporation and amino sugars separated by HPLC on a Dionex CarboPac PA1 analytical column (4×250 mm) with pulsed amperometric detection using a gold electrode.

STATISTICAL ANALYSIS

Statistical analysis was initially by single factor analysis of variance (ANOVA), comparing the six experimental groups for each measured variable, with results for inner and outer regions treated separately, followed by comparisons of means using a two-tailed Student's *t* test. Statistical significance was taken as $P<0.05$.

Results

MORPHOLOGY

The appearances of the opened, untouched control knee joints were uniformly normal. The knee joints of the animals subjected to a sham operation revealed only the external,

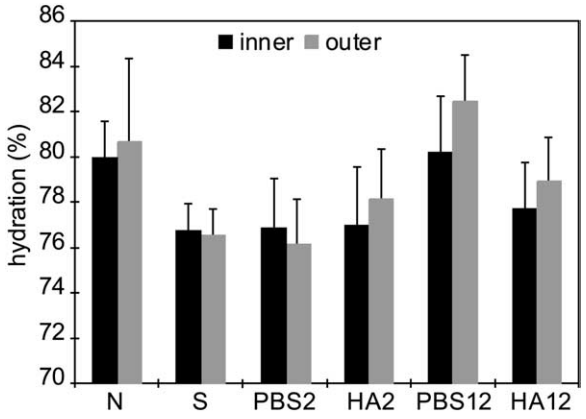

Fig. 1. Hydration (water content) of articular cartilage from the medial tibial condyle (inner and outer regions) in the six experimental groups. Error bars indicate one standard error of the mean. N: normal controls; S: sham operated animals; PBS2: animals injected for 2 weeks with intra-articular phosphate buffered saline; HA2: animals injected for 2 weeks with intra-articular sodium hyaluronate; PBS12: animals injected for 12 weeks with intra-articular phosphate buffered saline; HA12: animals injected for 12 weeks with intra-articular sodium hyaluronate.

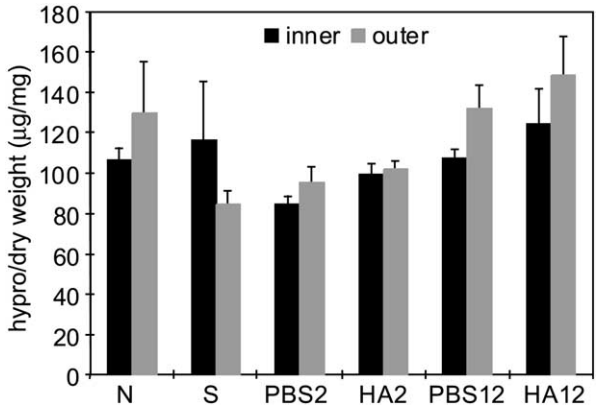

Fig. 2. Hydroxyproline content of articular cartilage (per tissue dry weight) from the medial tibial condyle (inner and outer regions) in the six experimental groups. Error bars indicate one standard error of the mean.

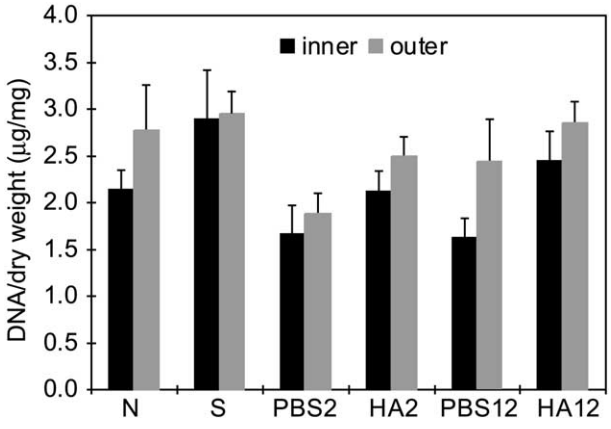

Fig. 3. DNA content of articular cartilage (per tissue dry weight) from the medial tibial condyle (inner and outer regions) in the six experimental groups. Error bars indicate one standard error of the mean.

sutured surgical incisions together with slight oedema of the subcutaneous connective tissue. In the case of the animals examined 6 weeks after surgery, the soft tissues around the knee joint were still slightly swollen. Within the joints, occasional orange coloration of the synovia confirmed the bleeding that had taken place into the joints when the capsules were incised. The greater part, but never all, of the medial menisci had been removed. The bearing surfaces of the lateral condyles did not differ from normal. Sixteen weeks after operation, rare flecks of white, granular material were seen on the surfaces of the medial condyles and the cartilage was often softer than normal. There were no significant differences in appearance between the joints of animals injected with PBS and those receiving HA.

BIOCHEMICAL COMPOSITION

The results (Figs. 1–6) include data from both inner (thick, medial) and outer (thin, lateral) regions of the tibial medial condyle. For each experimental group, results are shown in the following order: (i) normal, unoperated (N), (ii) sham operated (S), (iii) operated controls, injected with PBS for two weeks (PBS2), (iv) operated and injected with HA for two weeks (HA2), (v) operated controls, injected with PBS for 12 weeks (PBS12), and (vi) operated and injected with HA for 12 weeks (HA12).

There were no significant differences in hydration, compared to normal, in any of the experimental groups, either in the inner or outer regions of the medial condyle (Fig. 1). It is notable, however, that in both regions the highest water content was observed in the operated controls injected with PBS for 12 weeks (i.e. 16 weeks after surgery) while in the corresponding HA injected samples, water content was lower. There were also no significant differences in collagen content (measured as hydroxyproline per dry weight), compared to normal, in any of the experimental groups (Fig. 2). In the inner region only, DNA content in the PBS injected control groups, after both two weeks and 12 weeks, was lower than normal, unlike the HA injected groups, though these differences were not significant (Fig. 3). There were no significant differences in hydration, hydroxyproline or DNA content between normal and sham-operated controls, in both inner and outer regions.

When expressed per dry weight of cartilage (Fig. 4), the amounts of uronic acid in normal and sham operated samples were similar, in both inner and outer regions. After 2 weeks of injections (i.e. 6 weeks after surgery), amounts in operated controls (injected with PBS) were significantly lower than normal ($P<0.001$, for both inner and outer regions), as was the amount of uronic acid in the outer region of the HA injected group ($P<0.01$), though the uronic acid content of the inner region of the HA injected group appeared normal. After 12 weeks of injections (i.e. 16 weeks after surgery), there was an increase in uronic acid content in the PBS injected samples from the inner region, compared to normal ($P<0.02$), though this was not observed in the corresponding HA injected samples. In the outer region, the amount of uronic acid (per dry weight) in the PBS injected group was indistinguishable from normal, though the amount in the corresponding HA injected group

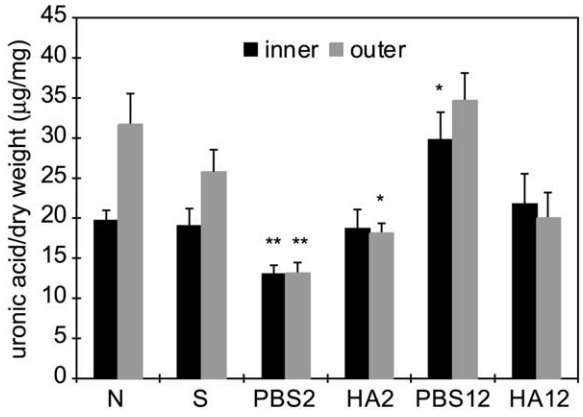

Fig. 4. Uronic acid content of articular cartilage (per tissue dry weight) from the medial tibial condyle (inner and outer regions) in the six experimental groups. Error bars indicate one standard error of the mean, and significant changes (compared to normals) are indicated (*P<0.02; **P<0.001).

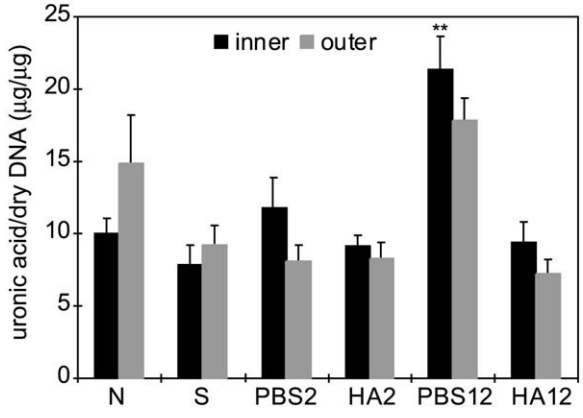

Fig. 5. Uronic acid content of articular cartilage (per µg DNA) from the medial tibial condyle (inner and outer regions) in the six experimental groups. Error bars indicate one standard error of the mean, and significant changes (P<0.001, compared to normals) are indicated (**).

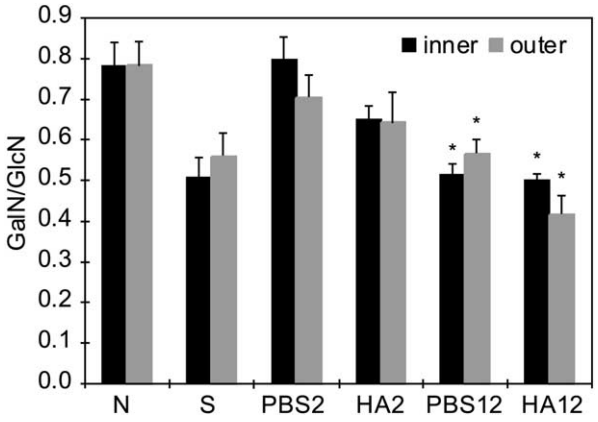

Fig. 6. Galactosamine/ glucosamine ratio in articular cartilage from the medial tibial condyle (inner and outer regions) in the six experimental groups. Error bars indicate one standard error of the mean, and significant changes (P<0.01, compared to normals) are indicated (*).

was low (P=0.03, compared to normal). It should be noted that the increases in uronic acid after 12 weeks were observed in the operated controls (injected with PBS), rather than the HA injected samples, despite the fact that uronic acid is a constituent of HA.

When uronic acid was expressed per total DNA (Fig. 5), the decrease observed in Fig. 4 in the operated controls after 2 weeks PBS injections was no longer apparent. The major observation was a large increase in the amount of uronic acid after 12 weeks with PBS, particularly in the inner region (P<0.001, compared to normal), which was not seen in the HA injected samples.

The ratio of galactosamine to glucosamine after 12 weeks of injections was significantly lower than normal (P<0.01, in both inner and outer regions), though there were no differences between the PBS and HA injected groups (Fig. 6).

Discussion

CHANGES IN ARTICULAR CARTILAGE COMPOSITION

Biochemical changes occurring during OA represent more than the mere passive degeneration of the articular cartilage. For example, loss of/damage to the cartilage in human OA is associated with increased synthesis as well as increased degradation of collagens, such that total amounts remain essentially unchanged[37]. Similar observations have been made in a number of rabbit models, including joint immobilization[38], total meniscectomy[27] and the partial meniscectomy model used here[23]. Our observations that net collagen levels remained essentially unchanged throughout the study are consistent with this earlier work, though any changes in rates of synthesis and degradation were not addressed here.

Though proteoglycan loss is a characteristic feature of long term cartilage degeneration, transient increases in proteoglycan levels, as observed here 16 weeks after surgery, are frequently observed. Increased proteoglycan synthesis rates have been reported in human OA[39], though there are considerable anatomical variations in this parameter[40]. There are also anatomical variations in total uronic acid content, as in the canine femoral condyle where the uronic acid content (per tissue dry weight) of unloaded regions is 20% lower than in loaded regions, while both water and DNA content are relatively uniform[41]. In the Pond-Nuki dog model of OA, McDevitt and Muir[14] reported no change in uronic acid levels in tibial articular cartilage up to 9 weeks after surgery, but increased levels (per tissue dry weight, compared to unoperated controls) after 48 weeks. Others[16,17], using the same dog model, also found increases in proteoglycan content, indicative of hypertrophic repair, followed (after 45 months) by cartilage loss. An increase in uronic acid content (per tissue dry weight) in the articular cartilage of the tibial plateau was also reported[21] after 6 weeks immobilization of the rabbit knee.

In the rabbit partial meniscectomy model, the rate of proteoglycan synthesis is maximal 12 weeks after surgery[24], even though (in PBS injected joints) there appears to be a reduction in the total amount of uronic acid (per

tissue wet weight) at this time point[42]. After total meniscectomy in rabbits, Hoch *et al.*[43] reported a fall in uronic acid content, compared to the non-operated contralateral joint, two weeks after surgery, which gradually recovered to normal levels after 26 weeks. The results reported here appear to conflict with this earlier work, since we observed a marked increase in uronic acid content (per tissue dry weight) 16 weeks after surgery in the PBS injected group. Several factors might account for this variation. First, we observed a reduction in uronic acid content 6 weeks after surgery, followed by an increase after 16 weeks. Thus the timing of the tissue sampling is important. Second, results expressed per tissue wet weight can be misleading if there are also changes in water content, as suggested by the results in Fig. 1. Third, the use of the non-operated contralateral joint can also be misleading, since altered load bearing and/or systemic factors can also give rise to biochemical changes in the articular cartilage. Fourth, the progression of joint degeneration may be influenced by differences in the surgical procedure.

The ratio of galactosamine to glucosamine in articular cartilage extracts is a measure of the chondroitin sulphate/keratan sulphate ratio. Increased galactosamine/ glucosamine ratios have been reported in the Pond-Nuki dog model of OA[14]. Similar observations have been reported in the rabbit meniscectomy model[27], where the galactosamine/glucosamine ratio in the medial tibial plateau reaches a maximum 5 weeks after surgery, falling thereafter. The observed fall in the galactosamine: glucosamine ratio between 6 weeks after 16 weeks after surgery (Fig. 6) is therefore consistent with earlier observations.

EFFECTS OF HA INJECTIONS

Studies on the biochemical effects of intra-articular HA on articular cartilage composition have been carried out using the anterior cruciate ligament transection model in dogs[44,45] and rabbits[46–48], the rabbit immobilization[21] and partial meniscectomy[49] models and the sheep total meniscectomy model[50].

In the anterior cruciate ligament transection dog model, the known increase in proteoglycan extractability[14] appeared to be partially prevented by weekly injections of HA (MW 750 kDa)[44]. Also in dogs, uronic acid concentration increased 30–60% in PBS injected compared to non operated joints, while HA (MW 1500 kDa) injected joints showed a 10–30% reduction[45]. These results are similar to those reported here (Figs. 4 and 5), where HA injection appeared to prevent the increase in uronic acid content in PBS injected joints 16 weeks after surgery. Similarly, in the rabbit knee immobilization model[21] a single injection of high MW HA prevented the increase in uronic acid content (per tissue dry weight) in the tibial plateau after 6 weeks immobilization. In the sheep medial meniscectomy model[50], weekly intra-articular injections of HA led to reduced proteoglycan synthesis and increased turnover, and the effects were greater with high MW (2000 kDa) compared low MW (800 kDa) HA.

In addition to the increase in uronic acid content observed 16 weeks after surgery in cartilage from the PBS injected joints, we also observed a drop in uronic acid content (per mg dry weight) 6 weeks after surgery (Fig. 4). This drop also appeared to prevented by HA injection. This result is similar to that observed using a rabbit anterior cruciate ligament transection model, using low MW (800 kDa) HA, where the femoral condyle cartilage was analysed 9 weeks after surgery[46].

The possibility that PBS injection may exacerbate the effects introduced by partial meniscectomy must be considered. It has been reported that excessive use of saline during arthroscopic procedures can have a deleterious effect upon chondrocyte metabolism[51]. Most recent studies on the effects of intra-articular HA in animal models have used PBS injection as controls[19,22,45,47,49,50,52]. An exception, however, is the work of Yoshioka *et al.*[46], who compared the effects of HA injections with PBS injected and non-injected operated controls following anterior cruciate ligament transection in rabbits. Similar results were obtained with PBS injected and non-injected controls, thus indicating that HA injection had a real effect on preventing changes in cartilage composition.

HA has numerous biological effects[12,53–55], involving interactions with specific cellular receptors[56]. It mediates the production of inflammatory mediators, is an effective scavenger of free radicals and an inhibitor of leukocyte adhesion, chemotaxis and phagocytosis. In addition, it can modulate cell migration, proliferation, apoptosis, aggregation and differentiation. Exogenous HA can also up- or down-regulate synthesis of endogenous HA, depending on MW[57], and can prevent degradation and cytokine induced release of proteoglycans into the extracellular matrix[58]. Some or all of these phenomena may be involved in the observed effect of exogenous HA on proteoglycan content in this rabbit model of OA.

Acknowledgements

We thank Mr A. R. Johnstone for help with the weekly injections, Mrs E. C. Wyatt for technical assistance, Professor S. C. Fry and Ms J. Aitken for help with the hexosamine analysis, Dr J. M. Anderson-MacKenzie for discussions and Mrs E Assier for help with the statistical analysis.

References

1. Gardner DL. Pathological Basis of the Connective Tissue Diseases. London: Edward Arnold 1992.
2. Poole AR, Rizkalla G, Ionescu M, Reiner A, Brooks E, Rorabeck C, *et al.* Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. Agents Actions 1993; 39(Suppl.):3–13.
3. Balazs EA, Denlinger JL. Viscosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol 1993;20(Suppl. 39):3–9.
4. Brandt KD, Block JA, Michalski JP, Moreland LW, Caldwell JR, Lavin PT. Efficacy and safety of intra-articular sodium hyaluronate in knee osteoarthritis. ORTHOVISC Study Group. Clin Orthop 2001; 385:130–43.
5. Altman RD. Status of hyaluronan supplementation therapy in osteoarthritis. Curr Rheumatol Rep 2003; 5:7–14.
6. Brandt KD, Smith GN Jr., Simon LS. Intra-articular injection of hyaluronan as treatment for knee osteoarthritis-What is the evidence? Arthritis Rheum 2000;43:1192–203.
7. Parenti D, Murray CW. Treatment of intra-articular osteoarthritis of the knee with hylan G-F 20: comment on the article by Brandt *et al.* Arthritis Rheum 2001; 44:1470–1.

8. Moskowitz RW, Altman RD. Efficacy of intra-articular hyaluronan in the treatment of knee osteoarthritis: comment on the article by Brandt *et al*. Arthritis Rheum 2001;44:1471–3.
9. Brandt KD, Smith GN Jr., Simon LS. Efficacy of intra-articular hyaluronan in the treatment of knee osteoarthritis: comment on the article by Brandt *et al*-Reply. Arthritis Rheum 2001;44:1473–6.
10. Felson DT, Anderson JJ. Hyaluronate sodium injections for osteoarthritis-Hope, hype, and hard truths. Arch Intern Med 2002;162:245–7.
11. Altman RD, Moskowitz R. Hyaluronate sodium injections for osteoarthritis: the truth. Arch Intern Med 2002;162:2498–9.
12. Ghosh P, Guidolin D. Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: Are the effects molecular weight dependent? Semin Arthritis Rheum 2002;32:10–37.
13. Pond MJ, Nuki G. Experimentally induced osteoarthritis in the dog. Ann Rheum Dis 1973;32:387–8.
14. McDevitt CA, Muir H. Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. J Bone Jt Surg 1976; 58-B:94–101.
15. McDevitt C, Gilbertson E, Muir H. An experimental model of osteoarthritis; early morphological and biochemical changes. J Bone Jt Surg 1977;59-B:24–35.
16. Vignon E, Arlot M, Hartmann D, Moyen B, Ville G. Hypertrophic repair of articular cartilage in experimental osteoarthrosis. Ann Rheum Dis 1983; 42:82–8.
17. Adams ME, Brandt KD. Hypertrophic repair of canine articular cartilage in osteoarthritis after anterior cruciate ligament transection. J Rheumatol 1991; 18:428–35.
18. Brandt KD, Braunstein EM, Visco DM, O'Connor B, Heck D, Albrecht M. Anterior (cranial) cruciate ligament transection in the dog: a bona fide model of osteoarthritis, not merely of cartilage injury and repair. J Rheumatol 1991;18:436–46.
19. Yoshimi T, Kikuchi T, Obara T, Yamaguchi T, Sakakibara Y, Itoh H, *et al.* Effects of high-molecular-weight sodium hyaluronate on experimental osteoarthrosis induced by the resection of rabbit anterior cruciate ligament. Clin Orthop Rel Res 1994; 298:296–304.
20. Stoop R, Buma P, Van der Kraan PM, Hollander AP, Billinghurst RC, Meijers TH, *et al.* Type II collagen degradation in articular cartilage fibrillation after anterior cruciate ligament transection in rats. Osteoarthritis Cart 2001;9:308–15.
21. Olsen EB, Trier K, Jorgensen B, Brok KE, Ammitzboll T. The effect of hyaluronic acid on cartilage in the immobilized rabbit knee. Acta Orthop Scand 1991; 62:323–6.
22. Sakakibara Y, Miura T, Iwata H, Kikuchi T, Yamaguchi T, Yoshimi T, *et al.* Effect of high-molecular-weight sodium hyaluronate on immobilized rabbit knee. Clin Orthop 1994;299:282–92.
23. Moskowitz RW, Davis W, Sammarco J, Martens M, Baker J, Mayor M, *et al.* Experimentally induced degenerative joint lesions following partial meniscectomy in the rabbit. Arthritis Rheum 1973;16:397–405.
24. Moskowitz RW, Howell DS, Goldberg VM, Muniz O, Pita JC. Cartilage proteoglycan alterations in an experimentally induced model of rabbit osteoarthritis. Arthritis Rheum 1979;22:155–63.
25. Colombo C, Butler M, O'Byrne E, Hickman L, Swartzendruber D, Selwyn M, *et al.* A new model of osteoarthritis in rabbits. I. Development of knee joint pathology following lateral meniscectomy and section of the fibular collateral and sesamoid ligaments. Arthritis Rheum 1983;26:875–86.
26. Hulth A, Lindberg L, Telhag H. Experimental osteoarthritis in rabbits. Preliminary report. Acta Orthop Scand 1970;41:522–30.
27. Floman Y, Eyre DR, Glimcher MJ. Induction of osteoarthrosis in the rabbit knee joint: biochemical studies on the articular cartilage. Clin Orthop Rel Res 1980; 00:278–86.
28. Shapiro F, Glimcher MJ. Induction of osteoarthritis in the rabbit knee joint: histological changes following meniscectomy and meniscal lesions. Clin Orthop Rel Res 1980;147:287–95.
29. Ghosh P, Sutherland J, Bellenger C, Read R, Darvodelsky A. The influence of weight-bearing exercise on articular cartilage of meniscectomized joints. An experimental study in sheep. Clin Orthop 1990; 252:101–13.
30. Armstrong S, Read R, Ghosh P. The effects of intra-articular hyaluronan on cartilage and subchondral bone changes in an ovine model of early osteoarthritis. J Rheumatol 1994;21:680–8.
31. Hulth A. Experimental osteoarthritis: a survey. Acta Orthop Scand 1982;53:1–6.
32. Lipman JM. Fluorophotometric quantitation of DNA in articular cartilage utilizing Hoechst 33258. Anal Biochem 1989;176:128–31.
33. Bitter T, Muir H. A modified uronic acid carbazole reaction. Anal Biochem 1962;4:330–4.
34. Carney SL. Proteoglycans. In: Chaplin MF, Kennedy JF, Eds. Carbohydrate Analysis: a Practical Approach. Oxford: IRL Press 1986;97–141.
35. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961; 93:440–7.
36. Hardy MR, Townsend RR, Lee YC. Monosaccharide analysis of glycoconjugates by anion exchange chromatography with pulsed amperometric detection. Anal Biochem 1988;170:54–62.
37. Lippiello L, Hall D, Mankin HJ. Collagen synthesis in normal and osteoarthritic human cartilage. J Clin Invest 1977;59:593–600.
38. Videman T, Eronen I, Candolin T. [^{3}H]proline incorporation and hydroxyproline concentration in articular cartilage during the development of osteoarthritis caused by immobilization. A study *in vivo* with rabbits. Biochem J 1981;200:435–40.
39. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523–37.
40. Grushko G, Schneiderman R, Maroudas A. Some biochemical and biophysical parameters for the study of the pathogenesis of osteoarthritis: a comparison between the processes of ageing and degeneration in human hip cartilage. Connect Tiss Res 1989; 19:149–76.
41. Slowman SD, Brandt KD. Composition and glycosaminoglycan metabolism of articular cartilage from habitually loaded and habitually unloaded sites. Arthritis Rheum 1986;29:88–94.

42. Howell DS, Carreno MR, Pelletier J-P, Muniz OE. Articular cartilage breakdown in a lapine model of osteoarthritis. Clin Orthop Rel Res 1986; 213:69–76.
43. Hoch DH, Grodzinsky AJ, Koob TJ, Albert ML, Eyre DE. Early changes in material properties of rabbit articular cartilage after meniscectomy. J Orthop Res 1983;1:4–12.
44. Abatangelo G, Botti P, Del Bue M, Gei G, Samson JC, Cortivo R, *et al.* Intra-articular sodium hyaluronate injections in the Pond-Nuki experimental model of osteoarthritis in dogs. Clin Orthop Rel Res 1989; 241:278–85.
45. Smith GN Jr., Myers SL, Brandt KD, Mickler EA. Effect of intra-articular hyaluronan injection in experimental canine osteoarthritis. Arthritis Rheum 1998;41: 976–85.
46. Yoshioka M, Shimizu C, Harwood FL, Coutts RD, Amiel D. The effects of hyaluronan during the development of osteoarthritis. Osteoarthritis Cart 1997; 5:251–60.
47. Shimizu C, Yoshioka M, Coutts RD, Harwood FL, Kubo T, Hirasawa Y, *et al.* Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. Osteoarthritis Cart 1998;6:1–9.
48. Amiel D, Toyoguchi T, Kobayashi K, Bowden K, Amiel ME, Healey RM. Long-term effect of sodium hyaluronate (Hyalgan) on osteoarthritis progression in a rabbit model. Osteoarthritis Cart 2003;11:636–43.
49. Kobayashi K, Amiel M, Harwood FL, Healey RM, Sonoda M, Moriya H, *et al.* The long-term effects of hyaluronan during development of osteoarthritis following partial meniscectomy in a rabbit model. Osteoarthritis Cart 2000;8:359–65.
50. Ghosh P, Read R, Numata Y, Smith S, Armstrong S, Wilson D. The effects of intra-articular administration of hyaluronan in a model of early osteoarthritis in sheep. II. Cartilage composition and proteoglycan metabolism. Semin Arthritis Rheum 1993;22:31–42.
51. Reagan BF, McInerny VK, Treadwell BV, Zarins B, Mankin HJ. Irrigating solutions for arthroscopy. A metabolic study. J Bone Joint Surg Am 1983; 65:629–31.
52. Kikuchi T, Yamada H, Shimmei M. Effect of high molecular weight hyaluronan on cartilage degeneration in a rabbit model of osteoarthritis. Osteoarthritis Cart 1996;4:99–110.
53. Laurent TC, Laurent UBG, Fraser JRE. The structure and function of hyaluronan: an overview. Immunol Cell Biol 1996;74:A1–7.
54. Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. Curr Opin Cell Biol 2000;12:581–6.
55. Moreland LW. Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. Arthritis Res Ther 2003; 5:54–67.
56. Turley EA, Noble PW, Bourguignon LYW. Signaling properties of hyaluronan receptors. J Biol Chem 2002;277:4589–92.
57. Smith MM, Ghosh P. The synthesis of hyaluronic acid by human synovial fibroblasts is influenced by the nature of the hyaluronate in the extracellular environment. Rheumatol Int 1987;7:113–22.
58. Shimazu A, Jikko A, Iwamoto M, Koike T, Yan WQ, Okada Y, *et al.* Effects of hyaluronic acid on the release of proteoglycan from the cell matrix in rabbit chondrocyte cultures in the presence and absence of cytokines. Arthritis Rheum 1993;36:247–53.