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Treatment with calcitonin prevents the net loss of collagen, hyaluronan and proteoglycan aggregates from cartilage in the early stages of canine experimental osteoarthritis

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Summary

Objective: To evaluate the effect of calcitonin (CT) on the histology and biochemistry of articular cartilage from unstable operated and nonoperated knee in a canine model of experimental osteoarthritis (OA).

Methods: Eighteen dogs underwent anterior cruciate ligament transection (ACLT) of the right knee and were randomly distributed into three groups of six dogs each. From day-1 after surgery until sacrifice 84 days post-ACLT, each dog received a daily nasal spray that delivered the placebo, 100 units of CT or 400 units of CT. Histologic lesions were scored. Hyaluronan (HA) and antigenic keratan sulfate (AgKS) were quantified by enzyme-linked immunosorbent assays (ELISAs), whereas aggrecan molecules extracted under nondissociative conditions were characterized by velocity gradient centrifugation.

Results: All canine cruciate-deficient knees developed OA. At a daily dose of 400 units, CT had no effect on the size of osteophytes but significantly reduced the severity of cartilage histologic lesions in unstable knees. CT also enhanced the HA content as well as the size distribution and relative abundance of fast-sedimenting aggrecan aggregates in cartilage from both operated and nonoperated knees. On the other hand, in the CT-treated group, the cartilage content of AgKS increased in operated joints, but not in nonoperated joints.

Conclusions: Because CT delivered as a nasal spray markedly reduced the severity of most OA changes, both at the histological and biochemical level, this form of therapy may have benefits for humans who have recently experienced a traumatic knee injury, and as well as for dogs who spontaneously rupture their ACL.
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Key words: Calcitonin, Osteoarthritis, Aggrecan, Keratan sulfate, Collagen, Hyaluronan.

Introduction

Although it is generally believed that chondrocyte-mediated degradation of the cartilage matrix is a major pathologic change in osteoarthritis (OA)[1,2], it is also increasingly recognized that other joint components contribute to the progression of the disease process. Indeed, in patients with established OA of the knee, disease progression is associated with a sustained increase in serum levels of hyaluronan (HA), a marker of synovial proliferation and hyperactivity[3,4]. Further, in knee OA, increased uptake of technetium-labeled bisphosphonate, which correlates with synovial fluid markers of cartilage and bone turnover, predicts subsequent joint space narrowing and, hence, cartilage loss[5,6]. Therefore, the therapeutic target in OA should not be restricted to articular cartilage, but should encompass other joint components including bone.

Difficulty in obtaining joint tissue from human OA joints before the disease is far advanced has stimulated the creation of animal models that simulate the human condition. Although these models must always be viewed with caution, they nevertheless improve our understanding of parallel pathologic changes in humans and allow the identification of early and important features of joint degeneration. Among the many animal models of experimentally induced OA currently available, the resection of the anterior cruciate ligament in the dog is well established[7,8]. Joint instability appears to be the initiating factor in

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the induction of cartilage lesions that closely resemble those of natural canine OA.

Previous studies have pointed out that in canine experimental OA, cartilage biochemical changes occur before cartilage morphological changes[7] and include a reduction in the HA content of cartilage matrix, a reduction in the antigenic keratan sulfate (AgKS) content of aggrecan molecules, as well as the progressive disappearance of the fast-sedimenting link protein-rich aggrecan aggregates[9–11]. These findings are worth stressing since similar changes in the biochemical composition and in the supramolecular organization of proteoglycan (PG) aggregates have also been observed in human OA cartilage[12,13]. Therefore, as in canine experimental OA, calcitonin (CT) not only markedly reduces the urinary levels of deoxypyridinoline, a specific marker of bone resorption, but also causes significant decreases in the severity of the OA cartilage changes as well as in the circulating blood levels of HA[14]. The present investigation was undertaken to determine to what extent this hormone might prevent, at least in part, these important biochemical OA changes in cartilage from canine cruciate-deficient knees.

Material and methods

MATERIALS

Highly purified collagenase (form III) was from Advance Biofactures Corp. (New York, NY, USA). All other reagents were of analytical grade and supplied by Sigma–Aldrich–Fluka (Bornem, Belgium).

Eighteen sets of vials for nasal spray delivery were kindly provided by Novartis (Basel, Switzerland). Six sets contained a placebo, six other sets were conditioned to deliver 100 units of salmon CT per spray and the last six sets were conditioned to deliver 400 units of salmon CT per spray. The sets were randomly numbered from 1 to 18 and shipped to the investigators. The authors were blinded to the content of each set during the entire period of the study and they were only informed after having entered the results of all parameters under study in a computer database.

ANIMAL PROTOCOLS

Animal housing and handling were conducted according to the rules and regulations of the Belgian Federal State. A total of 18 beagle dogs, approximately 4 years of age, were used. Each dog was randomly assigned a number ranging from 1 to 18 and, after quarantine and conventional health measures, the anterior cruciate ligament of the right knee was severed as previously reported[3,9]. The dogs were allowed to exercise for at least 1 h per day and were sacrificed on day-84 after surgery. Beginning on day-1 post-surgery through the end of the study time period, each animal received a daily nasal spray from the set of vials corresponding to its identification number.

JOINT EXAMINATION AND CARTILAGE TISSUE SAMPLING

After careful opening of the joint, synovial fluid was collected from both the operated and nonoperated knee of each animal and the volume of fluid was measured. Osteophyte formation was graded by measuring the maximal width (in mm) of the spur on each femoral trochlear ridge and the mean value was recorded. Cartilage damage on the femoral condyles and tibial plateaus was graded using a dissecting microscope. The surface of each erosion was measured and expressed in mm^2 before summing to yield the total surface of erosions. The depth of cartilage erosions was graded on a scale of 0–4, where 0 represented normal appearance and 4, a cartilage erosion extending to the subchondral bone. Macroscopic changes in the joints were scored by two independent observers, who noted similar values. We report here, in each case, the mean of the two scores.

Immediately after scoring, the medial femoral condyle from both knees was removed en bloc, fixed in 10% buffered formalin containing 5% cetylpyridinium chloride and shipped to Dr J. M. Williams, who was blinded to the interventions. Each specimen was scored according to the histologic–histochemical scale of Mankin *et al.*[15].

To circumvent depth-related variations, the whole cartilage thickness was sampled from the lateral femoral condyles for biochemical analysis and from the tibial plateaus for the characterization of aggrecan molecules.

BIOCHEMICAL ANALYSES

Cartilage specimens dissected from femoral condyles were lyophilized and weighed to obtain dry weights. They were then digested with twice-crystallized papain (100 μg/ml) in 0.15 M sodium acetate, 20 mM cysteine HCl, pH 6.0, for 24 h at 60°C. After digestion, the materials were centrifuged at 12,000 × *g* for 15 min.

Aliquots of the digests were analyzed for hydroxyproline content using the method of Woessner[16]. The collagen content was then calculated from the hydroxyproline content, by multiplying the latter by 8.33. The PG and chondroitin sulfate contents were measured by the previously described dimethylmethylene blue assay[17], using purified preparations of bovine nasal purified PG monomers and chondroitin sulfate as standards.

Antigenic (Ag) KS and HA were quantified by well-characterized enzyme-linked immunosorbent assay (ELISA) procedures[18,19]. All measurements in the AgKS inhibition ELISA refer to equivalents of an International Standard, kindly donated by Drs M. B. Mathews and A. L. Horwitz, The University of Chicago, Chicago, IL, USA. The relative percentage of AgKS found in PGs was expressed as the ratio of the AgKS concentration (weight/volume) relative to the sum of the concentrations (weight/volume) of chondroitin sulfate and AgKS.

EXTRACTION, PURIFICATION AND CHARACTERIZATION OF AGGRECAN MOLECULES

After sampling cartilage from both the medial and lateral areas of tibial plateaus that are covered by menisci, slices of cartilage specimens were incubated at 37°C for 6 h with a highly purified collagenase (1000 units per 100 mg wet weight cartilage) and a mixture of protease inhibitors[20]. The solubilized aggrecan molecules were then purified by two successive equilibrium density gradient centrifugations in cesium sulfate and cesium chloride, respectively. The purified a-A1 aggrecan molecules were dialyzed against a buffer containing 50 mM sodium acetate, 10 mM sodium EDTA, 100 mM E-aminocaproic acid, 10 mM *N*-ethylmaleimide, 5 mM benzamidine HCl, 5 mM phenylmethylsulfonyl fluoride and 5 mM iodoacetamide, pH 5.8 (Buffer A). The aggrecan concentration was adjusted to 0.1–0.3 mg/ml in Buffer A prior to characterization by velocity gradient centrifugation. These low aggrecan concentrations

minimize the Johnston–Ogston effect, and, thus, avoid the otherwise necessary extrapolation to zero concentration. In this centrifugal method, the aggrecan preparations are centrifuged in a preformed cesium sulfate density gradient, and, at the end of centrifugation, the determination of the hexuronate distribution throughout the centrifuge tube allows the calculation of the differential distribution function $g(S) = dG(S)/ds$ as described previously[20,21]. This methodology appears very precise and efficient for the centrifugal characterization of aggrecan preparations.

STATISTICAL ANALYSIS

After entering all measured data in a computer database (statistical software Prism™ from GraphPad™, version 3.0, San Diego, CA), the randomization of the CT preparations was revealed by Novartis to allow the comparison of data obtained in the three groups of animals: the group having received the placebo, the group having received a daily dose of 100 units of salmon CT, and the group having received a daily dose of 400 units of salmon CT. Comparisons among the three groups involved the estimation of mean, standard deviation (SD), and analysis of variance followed by post hoc testing (Tukey). A P value of less than 0.05 was considered as statistically significant.

Results

MORPHOLOGICAL FINDINGS

Only small volumes of synovial fluid (0.1–0.3 ml) were collected from the nonoperated knees of the animals in all three groups. Higher volumes were obtained from the operated knees (Fig. 1, upper left panel), ranging from 1.2 to 2 ml (mean: 1.5 ml) in the placebo-treated-group, from 0.5 to 2 ml (mean: 1.1 ml) in the 100 units CT-treated group and from 0.5 to 1.2 ml (mean: 0.8 ml) in the 400 units CT-treated group. Although treatment with CT was associated with a reduction in synovial fluid volume, a statistically significant decrease was only detected in the group treated with 400 units of CT when compared to the placebo group ($P < 0.05$).

The nonoperated knee joints from animals in all three groups were macroscopically normal. In the operated knees, the width of femoral osteophytes was distributed over a wide range of values in the three groups: 3–7 mm in the placebo-treated group (mean: 4.5 mm), 2–6 mm (mean: 3 mm) in the 100 units CT-treated group and 2–6 mm (mean: 3.5 mm) in the 400 units CT-treated group. No statistically significant differences were found between the three groups.

On the other hand, treatment with CT significantly reduced the mean size of both femoral condyle and tibial plateau cartilage ulcerations (Fig. 1, upper right and lower left panels, respectively). Furthermore, in the tibial plateau, but not in the femoral condyles, the mean size of ulcerations was significantly smaller in the group treated with 400 units of CT than in the group treated with 100 units of the hormone ($P < 0.05$). The grade of cartilage erosion seen in the femoral condyles and tibial plateaus ranged from 1 to 2 (mean: 1.5) in the placebo-treated group and was scored as 1 in all the animals treated with 400 units of CT (data not shown).

Medial femoral condyle specimens from the operated knees of the placebo-treated group all exhibited histological changes compatible with OA. As shown in Fig. 1 (lower right panel), the operated knee Mankin score was, however, significantly reduced in both the 100 units ($P < 0.001$) and 400 units CT-treated groups ($P < 0.001$) when compared to the placebo-treated group. There was no statistically

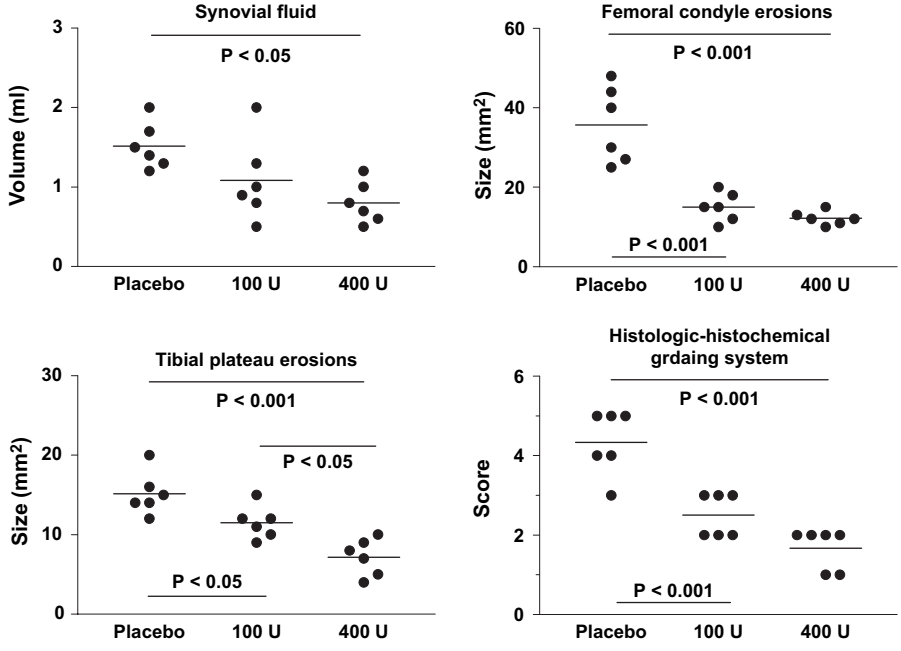

Fig. 1. Macroscopic and microscopic findings in the operated knees from animals treated daily with either the placebo, 100 units (U) of calcitonin or 400 U of calcitonin, and sacrificed on day-84 after surgery. The histologic–histochemical scale of Mankin was assessed on cartilage sampled from the medial femoral condyle. The horizontal lines represent mean values. The P value was determined by the Tukey's test following ANOVA.

significant difference in the mean Mankin scores of the operated knees between the two groups treated with 100 and 400 units of CT. In contrast, no histologic abnormality could be detected in the femoral condyle specimens from nonoperated knees in all three groups (data not shown).

BIOCHEMICAL FINDINGS

As stressed by Maroudas[22], wet weight determinations of OA cartilage can be misleading because cartilage slices lose water relatively easily when exposed to air. Further, in contrast to normal articular cartilage, OA cartilage swells considerably when equilibrated in physiologic saline solutions. Accordingly, the collagen, PG and HA contents all were expressed per unit dry weight (Fig. 2) when comparing the concentrations of these molecules in cartilage specimens from operated and nonoperated knees.

Comparisons between the placebo-treated group and the two CT-treated groups disclosed that, in cartilage sampled from the lateral femoral condyle, animals treated with CT exhibited a significant increase in the mean collagen content of their operated knees, all of which developed OA (Fig. 2, upper panel), but not of their nonoperated control knees. However, even at a daily dose of 400 units, the hormonal treatment was unable to eliminate the statistically significant difference in the mean collagen content of cartilage specimens from operated OA joints and nonoperated joints ($P < 0.05$) (Fig. 2, upper panel).

In the placebo-treated group, the cartilage sampled from the operated knees, all of which developed OA at 12 weeks post-surgery, exhibited a higher PG content than the cartilage sampled from the nonoperated knees ($P < 0.05$; Fig. 2, middle panel) whereas in the two groups treated with CT, there was no statistically significant difference in the mean PG content of cartilage between the operated and nonoperated knee joints. Further comparison between the PG content of cartilage from operated and nonoperated knees disclosed that, at a daily dose of either 100 or 400 units, CT enhanced significantly the mean PG content of nonoperated knees ($P < 0.05$) but had no effect on the mean PG content of operated knees.

At a daily dose of 100 units, CT had little effect on the mean HA content in cartilage sampled from the lateral femoral condyle of either operated OA or nonoperated knees, whereas, at a daily dose of 400 units, the mean HA content was significantly enhanced in cartilage sampled from both operated OA and nonoperated knees (Fig. 2, lower panel). It is worth noting that cartilage specimens from the operated OA knees of the 400 units CT-treated group exhibited HA contents (mean and range of values: 1.5; 1−2 μg/mg tissue dry weight, respectively) similar to those observed for the cartilage tissue from the nonoperated knees of the placebo-treated group (mean and range of values: 1.6; 1.1−2.1 μg/mg tissue dry weight, respectively) (Fig. 2, lower panel).

In the placebo-treated group, the mean AgKS content of PGs present in cartilage sampled from the lateral femoral condyle of operated OA knees was significantly lower than the mean AgKS content of PGs present in cartilage sampled from the same region of nonoperated knees (Fig. 3). On the other hand, treatment with CT was unable to change the mean AgKS content of PGs sampled from the

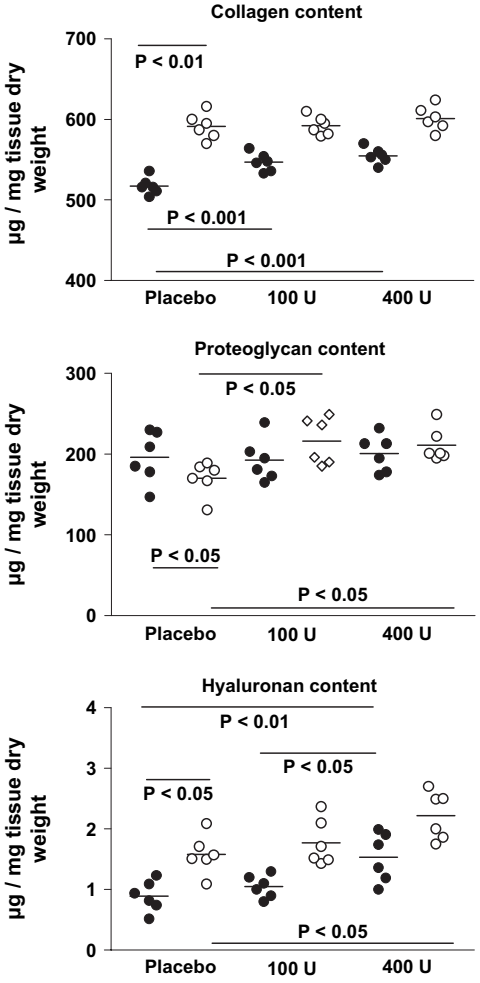

Fig. 2. Parameters of the biochemical composition of cartilage specimens sampled from the lateral femoral condyle of the operated knees (●, closed circles), all of which developed OA, and the nonoperated knees (○, open circles) of placebo- and calcitonin (CT)-treated [100 or 400 units (U)] dogs. Each point represents the mean of three determinations made on a given animal. In each scatter plot, the horizontal line represents the mean value. The *P* value was determined by the Tukey's test following ANOVA.

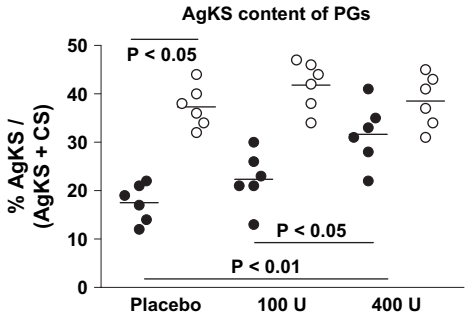

Fig. 3. Relative content of antigenic keratan sulfate (AgKS) of cartilage proteoglycan (PG) molecules, expressed as the ratio of AgKS to total sulfated glycosaminoglycans (chondroitin sulfate (CS) + AgKS), which were sampled from the operated OA (●, closed circles) and nonoperated (○, open circles) knees of animals treated daily with either the placebo, 100 units (U) or 400 U of calcitonin. Each point represents the mean of three determinations made on a given animal. The horizontal line represents the mean value. The *P* value was determined by the Tukey's test following ANOVA.

nonoperated knees whereas the hormone, at a daily dose of 400 units, enhanced markedly the mean AgKS content of PGs sampled from operated OA knee joints. It is worth stressing that, in the 400 units CT-treated group, the mean AgKS content of PGs was similar in both operated OA and nonoperated joints (25% vs 28.5%, respectively) and amounted to a value close to that observed for PGs sampled from the nonoperated knees of the placebo-treated group.

CENTRIFUGAL CHARACTERIZATION OF THE PG MOLECULES RECOVERED UNDER NONDISSOCIATIVE CONDITIONS FROM CARTILAGE OF OPERATED AND NONOPERATED JOINTS

Centrifugal studies were restricted to the cartilage PGs recovered from the tibial plateaus of dogs that were treated daily with the placebo or 400 units of CT.

As previously observed[12], pretreatment of cartilage specimens with highly purified collagenase in the presence of selected protease inhibitors yielded, in low ionic strength solutions, 60% to 70% of the total tissue hexuronate. After purification by two successive equilibrium density gradient centrifugations in different cesium salts, the nondissociated (a-A1 preparations) PG molecules recovered from the different cartilage specimens were characterized by velocity gradient centrifugation. In each group, the sedimentation profiles observed for the different a-A1 preparations of operated and nonoperated joints were averaged to generate the representative $g(S)$ distribution curves shown in Fig. 4. In all a-A1 specimens, the PG molecules were distributed into three well-defined sedimenting modes: the monomers (PG-M), with an average sedimentation coefficient of 16 S, the intermediate size or slow-sedimenting (PG-A1) aggregates ranging from 35 to 60 S and the more saturated or fast-sedimenting (PG-A2) aggregates ranging from 60 to about 120 S.

In the placebo-treated group, the sedimentation profile of a-A1 preparations from operated OA joints (Fig. 4, upper right panel) differed markedly from that of a-A1 preparations from nonoperated joints (Fig. 4, upper left panel). In agreement with previous studies[12,14], the a-A1 preparations from operated OA joints contained predominantly monomers ($P < 0.05$). Further comparison between the a-A1 preparations from operated and nonoperated joints showed that, in preparations from operated joints, the proportion of PG-A1 aggregates was significantly reduced (mean: 36% vs 46% for nonoperated joints; $P < 0.05$) whereas their PG-A2 aggregates were consistently smaller (mean: 70 S vs 87 S for nonoperated joints; $P < 0.05$) and less abundant (mean: 7% vs 22% for nonoperated joints; $P < 0.05$).

In contrast, in the CT-treated group (400 units), the differences in the sedimentation profile between the a-A1 preparations recovered from operated OA knees (Fig. 4, lower right panel) and the a-A1 preparations recovered from nonoperated knees (Fig. 4, lower left panel) were less striking. In both preparations, the majority of PG molecules were present as aggregates. However, in preparations from nonoperated joints, the mean percentage of PG-Ms was significantly reduced (25% vs 41%; $P < 0.05$) whereas the mean percentage of PG-A1 was significantly increased (48% vs 41%; $P < 0.05$). Further comparison between a-A1 preparations from both joints disclosed that the PG-A2

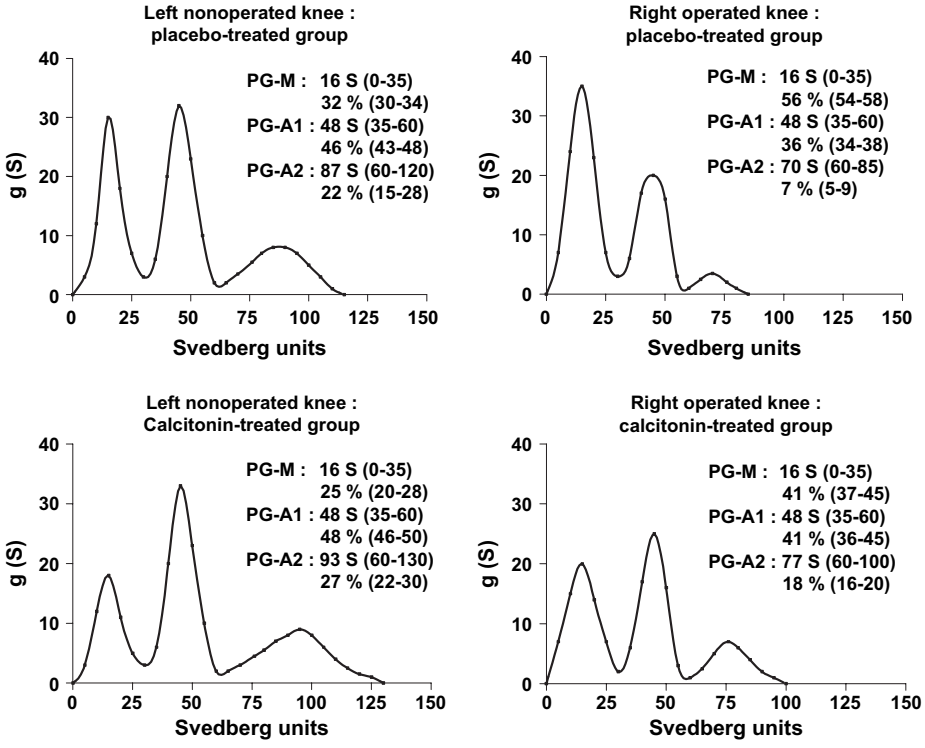

Fig. 4. Averaged centrifugal distribution curves from results of boundary sedimentation analyses of cartilage proteoglycans (PGs) recovered under nondissociative conditions from nonoperated and operated OA knees of dogs treated daily with either the placebo or 400 units of calcitonin (CT) and then sacrificed on day-84 after surgery. For each curve and from left to right, the first peak represents the PG monomers (PG-M), the second peak represents the intermediate size or slow-sedimenting (PG-A1) aggregates and the third peak represents the more saturated or fast-sedimenting (PG-A2) aggregates (range of values).

aggregates were larger (mean: 93 S vs 77 S; $P < 0.05$) and more abundant (mean: 27% vs 18%; $P < 0.05$) in preparations from nonoperated knees.

Obviously, treatment with CT induced marked changes in the sedimentation profile of a-A1 preparations from both nonoperated and operated OA joints. Indeed, in both joints, administration of the hormone led to a decrease in the mean abundance of PG-Ms ($P < 0.01$ for both joints) and concomitantly enhanced the mean percentage of PG-A1 aggregates ($P < 0.05$ for nonoperated joints and $P < 0.01$ for operated OA joints). Further, although the hormone treatment was associated with an increase in both the mean size and mean percentage of PG-A2 aggregates from both knees, a statistically significant difference between placebo- and CT-treated groups was only observed in the operated OA knees for both the mean size ($P < 0.01$) and the mean percentage ($P < 0.01$).

Discussion

The results of the double-blinded prospective study presented here indicate that, in the early stages of canine experimental OA, the daily nasal administration of 400 units of CT markedly slowed down the development of most cartilage degenerative changes. The order of magnitude of biochemical and morphologic changes observed in cartilage from operated OA knees is similar to that observed in previous studies at a similar time period post-surgery[7,9–13].

These results not only confirm a previous report showing that in the same animal OA model the subcutaneous administration of CT significantly reduced the severity of cartilage microscopic OA changes[14], but they also clearly demonstrate that the hormonal treatment was accompanied by striking changes in the biochemical composition and supramolecular organization of articular cartilage from OA knees. The fact that the treatment with CT was effective in reducing the net loss of HA and collagen associated with the development of OA changes is, by itself, a finding of singular significance. Given the critical functional properties of aggrecan molecules that populate cartilaginous structures, the discovery that this treatment also was successful in reducing the increase in the relative proportion of aggrecan monomers as well as the concomitant decrease in the relative proportion of PG aggregates of large sizes is likely to be of great interest to both clinicians and scientists. Although, for practical reasons, biochemical determinations were conducted on cartilage from femoral condyles and centrifugal analyses were restricted to PGs extracted from tibial plateaus, our findings remain meaningful because in canine experimental OA, changes in gene expression and the biochemical composition of articular cartilage occur throughout the entire compartment of the OA joint[7–11,24]. In contrast to its marked effects on the biochemical composition and supramolecular organization of cartilage, CT, a potent inhibitor of bone osteoclastic resorption, had no effect on osteophyte formation. This lack of pharmacologic effect on osteophytosis should not be surprising since osteophyte formation involves bony metaplasia of new fibrous connective tissue and is not reliant on bone resorption.

CT added to the medium in which articular chondrocytes are cultured enhances PG synthesis in a dose-dependent manner[25]. In our animal model of OA, treatment with CT caused an increase in PG content of articular cartilage, but this was limited to the nonoperated joint examined. The failure of CT to enhance the PG content of cartilage from operated OA joints might reflect, at least in part, the fact that chondrocytes in these joints already are maximally stimulated to synthesize these functional matrix molecules. This stimulation of PG synthesis, which is sustained for as long as 64 weeks after surgery[8,23], usually overcompensates for increased PG degradation and leads to the increase in the PG content of OA cartilage seen in this study and other reports[11,23]. This phenomenon, termed hypertrophic repair, is not restricted to canine OA but has also been observed in other animal models of OA and in human OA as well, at least until the late stages of the disease process[26].

Further, although CT seems to have no effect on the PG-degrading activity secreted by chondrocytes[27], bone cells derived from human OA joints secrete an as yet undefined factor that influences the metabolism of cartilage explants[28]. Should such factors normally be sequestered within the bone matrix *in vivo*, the CT-induced suppression of osteoclastic bone resorption would have inhibited the release of this factor.

Despite its enhanced PG content, hypertrophic OA cartilage does not withstand mechanical stress as well as normal hyaline cartilage[29,30] and full-thickness loss of cartilage tissue develops with time post-surgery[8]. These observations may be related, at least in part, to the reduction in content of HA[9,12], the backbone upon which aggrecan molecules align to form aggregates, and to the progressive depletion of the fast sedimenting or more saturated PG aggregates[10,13] seen in canine OA cartilage and human OA cartilage as well. HA not only helps to firmly immobilize PGs at high concentrations within the collagen network, but it also dramatically increases the rheological properties of PG molecules[31]. Accordingly, the reduction in HA content, accompanied by a reduction in the relative abundance and size distribution of PG aggregates is likely to markedly reduce the viscoelastic properties of articular cartilage and, in so doing, to contribute to the apparent irreversibility of the OA disease process. The key contributory role that the decrease in HA content may play in OA progression is exemplified by the fact that the content of HA in articular cartilage remains normal in disuse atrophy, a condition in which the chondrocytes are able to overcome the loss of PGs that ensues as a result of increased enzyme activities[11].

The mechanisms responsible for the HA depletion seen in OA cartilage remain unclear. As the loss of HA from OA cartilage explants occurs in spite of an upregulation in HA biosynthesis[32,33], a likely explanation is that HA strands are being degraded at an accelerated rate by a hyaluronidase. The problem is that no hyaluronidase activity at near neutral pH has been thus far identified in normal and OA cartilage. An alternative explanation is that the decrease in HA content is connected to an increase in internalization of HA and its degradation to small oligosaccharides within a low pH lysosomal compartment, with the endocytosis being mediated via cell surface CD44/HA receptors[34].

In CT-treated dogs, the HA content of cartilage remained higher in the nonoperated joints than in operated OA joints, suggesting that the hormone was unable to completely inhibit the mechanisms responsible for the relative depletion of HA in OA cartilage. This notwithstanding, the results also showed that by enhancing the HA content in cartilage from both operated OA and nonoperated canine knees, CT increased the relative amounts of PG aggregates observed in both joints. One may postulate, therefore, that by improving the resistance of the articular tissue to the considerable stresses and strains arising from joint loading,

CT treatment contributed to the observed reduction in the score of the OA microscopic lesions.

Measurement of the AgKS content of aggrecan monomers revealed that the administration of CT also appeared to affect the quality of the PGs synthesized by chondrocytes in the operated OA joint. The fact that the content of AgKS in cartilage within the operated OA joint of animals treated with CT did not decrease, as it did in animals not receiving the hormone, is interesting. Our original observation that the content of AgKS in aggrecan decreases with the development of degenerative changes in this canine model of OA[10] led us to suggest that these chondrocytes switch to the synthesis of an ''immature'' PG, which resembles the chondroitin sulfate-rich aggrecan molecules produced by fetal chondrocytes. The findings presented here, therefore, suggest that CT is able to influence not only the quality of the PG aggregates in OA tissue but also that of the aggrecan monomer synthesized by the cells. While the content of AgKS in aggrecan is not thought to vary markedly during adult life[18] one cannot rule out at this stage the possibility that these observed changes in AgKS content reflect, in part, a decrease in the size or sulfation of KS chains.

The disruption and remodeling of the collagen network is an early event in this model of canine OA. This is reflected by a decrease in collagen content and hydroxypyridinium crosslink density of the cartilage tissue[29,30]. In addition, there is also an increase in the cartilage content and synovial fluid levels of type II collagen neoepitopes that are generated by cleavage of the alpha chains by any one of the three collagenases (MMP-1, MMP-8 and MMP-13), which are all produced by chondrocytes[35,36]. The cleavage and denaturation of type II collagen, the most abundant collagen of cartilage matrix, is likely to be a major event in the pathophysiology of the OA process. Damage to the collagen network is believed to be difficult to repair and, when significant, is thought to herald irreversible progress toward articular cartilage destruction. Accordingly, our finding that CT reduced a net loss of collagen from cartilage of OA knees may be of great clinical importance. It is supported by previous *in vitro* studies[25,27] showing that the hormone not only increases the production of type II collagen by human articular chondrocytes, but also inhibits the collagenase activity present in the culture medium of OA chondrocytes either by decreasing the activation of procollagenases and/or by increasing the levels of tissue inhibitors of metalloproteinases (TIMPs).

In summary, the results of the microscopic and biochemical analyses demonstrate that the daily intra-nasal administration of 400 units of CT significantly reduces the severity of most OA changes in the cruciate-deficient knee. Accordingly, this form of treatment, which most likely targets different compartments of the joint, may be beneficial for humans who recently experienced a traumatic knee joint injury and for dogs that have spontaneously ruptured their anterior cruciate ligament.

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