

AMELIORATION OF LAPINE OSTEOARTHRITIS BY TREATMENT WITH GLYCOSAMINOGLYCAN-PEPTIDE ASSOCIATION COMPLEX (RUMALON)

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The chondroprotective potential of glycosaminoglycan-peptide association complex (GP-C) was examined in the medial meniscectomy model of lapine osteoarthritis (OA). Prophylactic treatment with increasing doses of intramuscular GP-C (0.05–0.5 ml/kg) caused a significant reduction in OA lesion area and histologic scores, and the effect on disease activity appeared to be dose related. The DNA and uronic acid contents of OA tissue were unaffected by prophylactic treatment with GP-C. However, levels of hydroxyproline in OA cartilage in-

creased to near control levels with prophylactic treatment. Cartilage levels of active and total metalloproteinases that digest proteoglycans were elevated in rabbits with OA; prophylactic treatment with low-dose GP-C (0.05 ml/kg) produced a significant reduction in active, but not total, enzyme. Cartilage levels of tissue inhibitor of metalloproteinases in animals with OA were comparable with control levels, but rose with increasing doses of GP-C. We also investigated GP-C as a therapeutic treatment in animals that had already developed OA lesions. Carbon black retention and histologic score returned to near-normal after therapeutic treatment with GP-C. Uronic acid and hydroxyproline levels were decreased in OA cartilage. Therapeutic treatment with GP-C had no statistically significant effect on uronic acid levels, but was associated with increased hydroxyproline content in the cartilage. The changes in metalloproteinase and metalloproteinase inhibitor were similar to those found in the studies of prophylactic treatment. The findings in this animal model may help explain some of the beneficial effects of GP-C in human OA.

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Supported by the Veterans Administration, NIH grant AR-08662, and a grant from Robapharm, Ltd., Basel, Switzerland.

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Submitted for publication March 29, 1990; accepted in revised form September 13, 1990.

Osteoarthritis (OA) is a common disorder that affects over 40 million Americans (1). It is characterized by loss of matrix proteoglycans (PG), fibrillation of the cartilage surface, and eventual loss of collagenous matrix to expose underlying bone (2). The etio-pathogenesis of OA has been difficult to determine but is probably related to mechanical wear, failure of the chondrocyte to maintain a balance of matrix synthesis and degradation, and extracartilaginous factors such as bony remodeling and synovium-mediated events (3).

Matrix proteoglycans play an important role in the structural integrity of cartilage. Mankin et al (4)

and Ehrlich (5) found a loss of matrix PG in cultured OA cartilage and suggested that degradative activity was elevated in these tissues. Enzymes that degrade PG at physiologic pH and belong to the metalloproteinase class have been identified in cartilage. Sapolsky et al (6) partially characterized two enzymes: one active at acid pH and the other at neutral pH. Woessner and Selzer (7), Gunja-Smith et al (8), and Azzo and Woessner (9) further characterized these two activities. Subsequent investigations have demonstrated increased levels of both enzymes in human and canine OA cartilage (10–14).

Cartilage also contains tissue inhibitor of metalloproteinases (TIMP) (15,16), which inhibits both of these metalloproteinases, as well as collagenase. In OA, TIMP has been found to be increased either modestly or not at all, while metalloproteinase levels are increased 3–5-fold (9,12,16). This would lead to an imbalance in which the proteinases escape control by the inhibitor, resulting in excessive matrix degradation.

Current therapy for patients with OA is primarily palliative and aimed at reducing pain, retarding inflammation, and maintaining joint function. Treatments currently in use have not been shown to modify the pathologic processes that occur in the tissue. Recently, a new approach has been advocated, in which the treatment aim is to stimulate cartilage repair and, at the same time, inhibit cartilage breakdown (17). This concept has been termed "chondroprotection." Prototypic drugs in this category have been described and subjected to clinical trials in Europe (18).

One drug believed to belong in the chondroprotective category is glycosaminoglycan-peptide association complex (GP-C) (Rumalon; Robapharm, Basel, Switzerland). GP-C is an aqueous extract of young mammalian (bovine) cartilage and bone marrow containing 2.5 mg glycosaminoglycan-peptide complex in 6.5 mg dry substance and 2.6 mg meta-cresol as preservative per ml. GP-C contains approximately 1.8 mg/ml glycosaminoglycans (mainly chondroitin-4-sulfate and chondroitin-6-sulfate) and 0.7 mg/ml peptides. There is evidence that this agent also contains amino acids and growth-stimulating factors that have the ability to increase the anabolic activity of cartilage in vitro (17,19,39).

The present study was undertaken first to determine whether development of OA lesions in the rabbit meniscectomy model could be blocked by treatment with GP-C. A second study using the same model was undertaken to determine if existing OA lesions could be repaired by GP-C treatment. The results of

both investigations provide evidence that GP-C does display the properties expected of a chondroprotective agent.

MATERIALS AND METHODS

Induction of OA. Osteoarthritis of the knee was induced by partial medial meniscectomy, as described by Moskowitz et al (20,21), in skeletally mature New Zealand white rabbits weighing 3 kg. The animals were anesthetized with a mixture of ketamine HCl (35 mg/kg) and xylazine (5 mg/kg) given intramuscularly. An anteromedial incision was made in the right knee, soft tissue and retinaculum were incised and reflected medially and laterally, and the joint capsule was opened to expose the medial aspect of the joint. One-half of the medial meniscus was freed and one-third excised with iris scissors. Incisions were closed with 5-0-coated Vicryl sutures. Restoration of normal eating habits and ambulation were evident within 1–3 days after the operation.

The operated rabbits were divided into groups that would receive prophylactic GP-C treatment, groups that would receive therapeutic GP-C treatment, and groups that would not receive GP-C treatment (OA controls). Age-matched groups of unoperated rabbits served as normal controls. Each group consisted of 7–10 animals.

GP-C treatment. The prophylactic GP-C treatment groups were treated from day 5 after surgery with intramuscular injections of 0.05–0.5 ml/kg GP-C 3 times per week, to determine if GP-C was able to block the development of OA lesions. The therapeutic treatment groups were left untreated for 6 weeks to allow OA lesions to develop, and then were treated with 0.25 ml/kg or 0.5 ml/kg GP-C intramuscularly 3 times per week for 14 weeks, to determine if the lesions could be repaired. Both the prophylactic and therapeutic dosage levels were suggested by the manufacturer based on earlier in vitro and in vivo studies, as reviewed by Burkhardt and Ghosh (17).

Cartilage from contralateral legs was not used for control tissue because pathologic changes were noted in early phases of the study. All animals were killed with 1.0 cc of T-61 euthanasia solution.

Gross pathologic and histologic study of cartilage. When each rabbit was killed, the knee joint was carefully opened and the femoral condyles examined for spur formation, pitting, and ulceration. Sketches were made to record the location and extent of gross findings. Some condyles were coated with carbon black as described by Meachim (22); the lesion area was measured under a dissecting microscope at 10× magnification, and the stained condyle was photographed to obtain a permanent record.

Small sections of cartilage from the weight-bearing area of the medial femoral condyle were fixed in 10% neutral buffered formalin, decalcified in Cal Ex II (Fisher Scientific, Fair Lawn, NJ), and embedded in paraffin. Alternate 5- μ m sections were stained with Safranin O-light green and graded for degree of OA (4). The remaining cartilage was dissected free of subchondral bone and stored in physiologic saline at –80°C until assayed.

Extraction and assay of cartilage proteinases. Cartilage (30–60 mg wet weight) was sliced into 25- μ m sections on a freezing microtome and then homogenized with a motor-driven ground-glass homogenizer (Duall #20; Kontes Glass, Vineland, NJ) in 20 volumes of ice-cold buffer containing 1.0M guanidine HCl, 50 mM Tris HCl, and 10 mM CaCl₂, pH 7.5. The homogenate was centrifuged at 10,000g for 20 minutes at 4°C. The enzyme-containing supernatant was then dialyzed against assay buffer (50 mM Tris HCl, 10 mM CaCl₂, 0.2M NaCl, 0.05% Brij 35, pH 7.5).

Proteoglycan degradation was assayed by the method of Nagase and Woessner (23), as modified by Woessner and Selzer (7). The assay is based on the digestion of ³H-PG monomer, which is entrapped in polyacrylamide gel beads. The pore size of the beads is adjusted so that the undigested monomers are retained inside the beads, while the digestion fragments of \leq 200,000 daltons are allowed to diffuse out. The assay measures the digestion of PG core protein and can detect \sim 0.3 ng trypsin in 2 hours at 37°C.

For assay, 0.1-ml enzyme samples were mixed with 0.1 ml of assay buffer and then added to a 7.0-ml glass scintillation vial containing \sim 2.0 mg ³H-PG-containing beads (12,000 counts per minute/mg beads; 300 μ g PG). The level of active enzyme was determined without any addition, while 1.0 mM APMA (ICN Biochemicals, Irvine, CA) was added to activate latent enzyme. Blanks were prepared by adding 1.0 mM 1,10-phenanthroline (Sigma, St. Louis, MO) to inhibit all metalloproteinase and 1.0 mM phenylmethylsulfonyl fluoride (Sigma) to inhibit all serine proteinase.

After 18 hours of incubation at 37°C, 6.0 ml of Aquasol scintillation fluid (New England Nuclear, Boston, MA) was added and the activity in the vials counted in a Tri-Carb Model 3003 scintillation counter (65% efficiency; Packard, Downers Grove, IL). Enzyme activity was expressed as enzyme units/gm wet weight tissue, based on 1 enzyme unit being equivalent to 1 μ g ³H-PG released per minute at 37°C.

Assay of TIMP. TIMP was measured with active collagenase extracted from the growth plate of rachitic rats (24,25), because enzymes in rabbit cartilage interfered with the Azocoll assay (Calbiochem-Behring, La Jolla, CA) used in earlier studies (15). Collagenase was activated by treatment with 0.5 mM APMA for 1 hour at 37°C and was then extensively dialyzed against assay buffer.

For assay, active collagenase (9 enzyme units; 1 enzyme unit = 1 ng collagen digested/minute at 30°C), inhibitor (0–20 μ l), and assay buffer (final volume 50–60 μ l) were mixed together and preincubated for 1 hour at 37°C to allow enzyme and inhibitor to react. After preincubation, 32 μ g of ³H-collagen (see below) and buffer sufficient to yield a final volume of 110 μ l were added, and incubation was continued for 22 hours at 30°C. Enzyme blanks contained 1 mM 1,10-phenanthroline. At the end of incubation, the standard collagenase assay was performed as described previously (26). One unit of inhibitor blocked 1 enzyme unit.

Determination of DNA, uronic acid, and hydroxyproline content. Cartilage samples (4–8 mg wet weight) were digested according to the method of Oegema et al (27), in 200 μ l 0.1M sodium phosphate buffer, pH 6.5, containing 0.01M EDTA and 100 μ g proteinase K (type XI from *Tritirachium album*; Sigma). After 18 hours at 56°C, the digests were

cooled to room temperature and then frozen at -20° C until assayed.

The DNA content of the digest was determined by the method of LaBarca and Paigen (28), using bisbenzimidazole. For uronic acid assays, the method of Bitter and Muir (29) was followed, with reduction of all reagent volumes to compensate for the relatively small volume of sample. The hydroxyproline content of the digest was determined by the method of Morales et al (30), followed by the colorimetric assay described by Woessner (31).

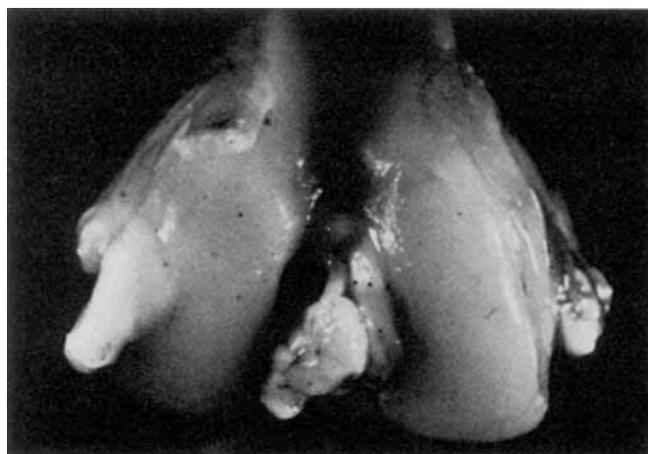
Statistical analysis. Means, standard deviations, and standard errors were calculated, and groups were compared using Student's *t*-test. *P* values less than 0.05 were considered significant (32).

RESULTS

The overall study was divided into two phases. The first phase was designed to test the ability of GP-C to block the development of OA lesions. In this way, the efficacy and dose-dependence of GP-C's effects on several well-known characteristics of OA tissues could be evaluated over a short period of time. The encouraging results of prophylactic treatment prompted a second phase of the study, in which animals were treated therapeutically with GP-C after OA lesions had developed. This segment of the study allowed the evaluation of GP-C's ability to induce repair of existing lesions.

Results of prophylactic treatment. The articular cartilage from unoperated, age-matched control animals displayed all the characteristics of normal rabbit cartilage. The surface was smooth, glistening, and white; no carbon black was retained when painted on the surface of the condyle (Figure 1A). Rabbits that had been subjected to partial medial meniscectomy and allowed free ambulation for 6 weeks developed lesions characteristic of OA. The cartilage surface was slightly roughened and dull, pitted, and yellowed in color. Carbon black was retained in regions of fissures and erosion (Figure 1B). Rabbits treated 3 times per week with 0.25 ml/kg GP-C showed reduced retention of carbon black and erosion, whereas those treated with 0.5 ml/kg GP-C had a significant reduction in erosion area compared with OA controls (mean \pm SEM 0.2 ± 0.1 mm² versus 0.9 ± 0.2 mm²; *P* < 0.01), although they still had pathologic changes that distinguished them from unoperated controls (Figures 1C and 3A).

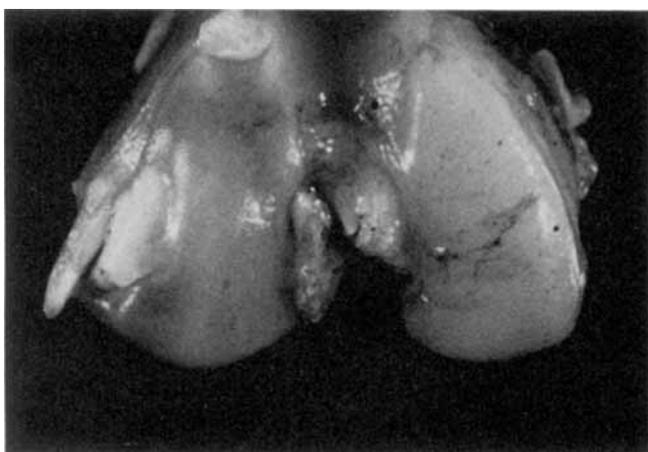
The histologic appearance of cartilage from the rabbits in the different study groups can be seen in Figure 2. Cartilage from unoperated, age-matched controls had a smooth surface, stained with Safranin O, and had normal cellularity and an intact tidemark



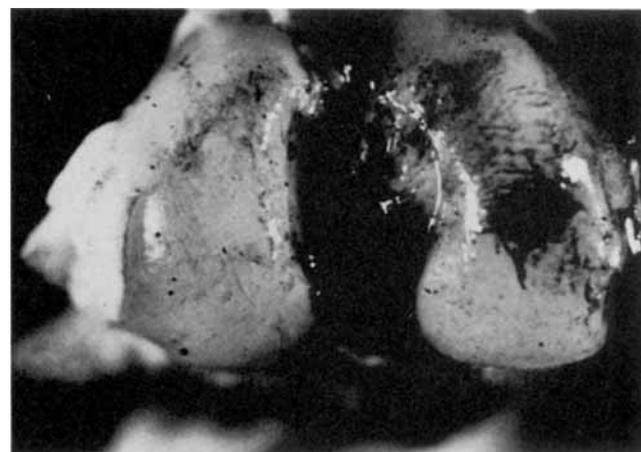
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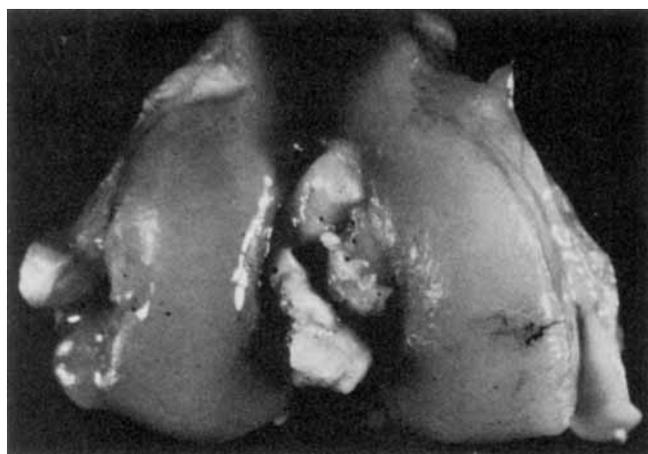
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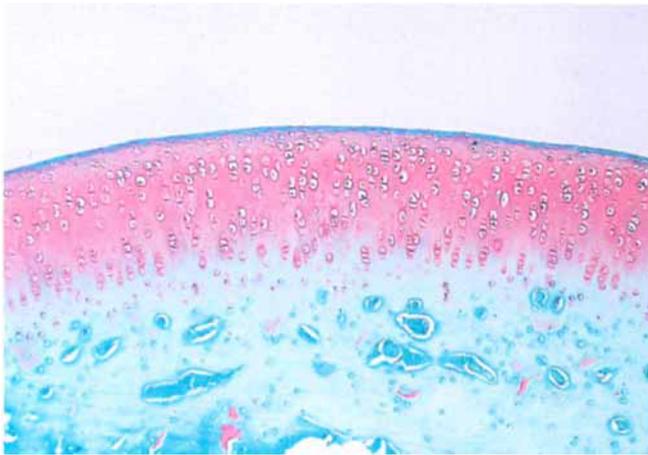


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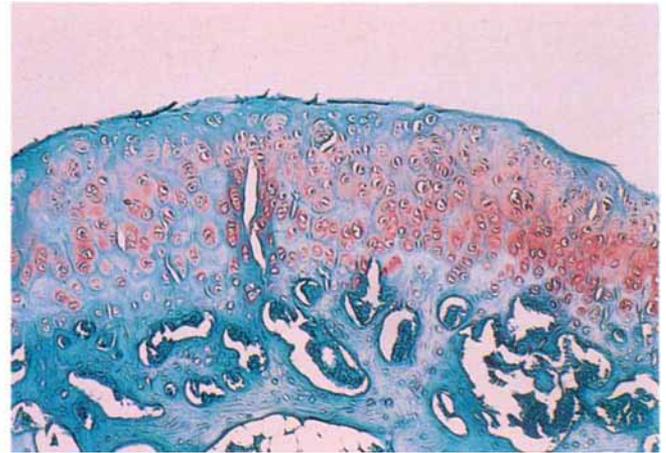
Figure 1. Carbon black staining of lapine femoral condylar cartilage. **A**, Distal femoral condyle of an unoperated rabbit (normal control, prophylactic treatment study), showing lack of staining. **B**, Distal femoral condyle of a rabbit 6 weeks after partial medial meniscectomy (osteoarthritis [OA] control, prophylactic treatment study). **C**, Distal femoral condyle of a rabbit that was subjected to partial medial meniscectomy and then treated with 0.25 ml/kg glycosaminoglycan-peptide association complex (GP-C) 3 times per week for 6 weeks (prophylactic treatment study). **D**, Distal femoral condyle of a rabbit 20 weeks after partial medial meniscectomy (OA control, therapeutic treatment study). **E**, Distal femoral condyle of a rabbit that was subjected to partial medial meniscectomy 20 weeks earlier and was allowed to develop an OA lesion over 6 weeks, before initiation of GP-C treatment (0.25 ml/kg 3 times per week for 14 weeks) (therapeutic treatment study). (Original magnification $\times 5$.)

(Figure 2A). OA cartilage was characterized by an irregular surface, partial loss of Safranin O staining, and loss of normal cellularity and tidemark integrity (Figure 2B). Treatment with 0.25 ml/kg GP-C 3 times

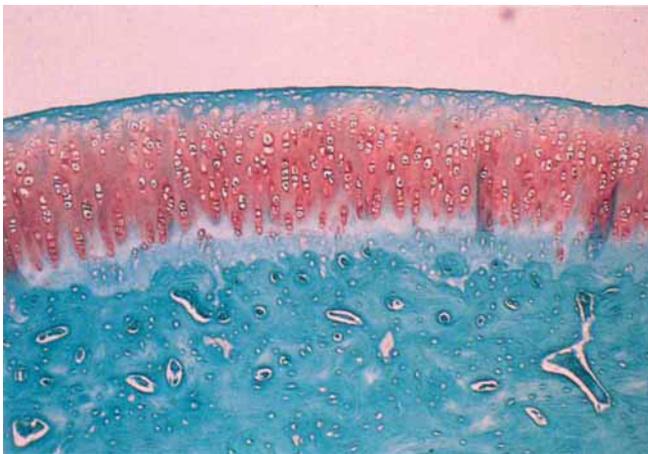
weekly resulted in histologic findings similar to those in unoperated controls. Surface irregularity was near-normal and Safranin O staining was partially retained, as were tidemark integrity and cellularity (Figure 2C).



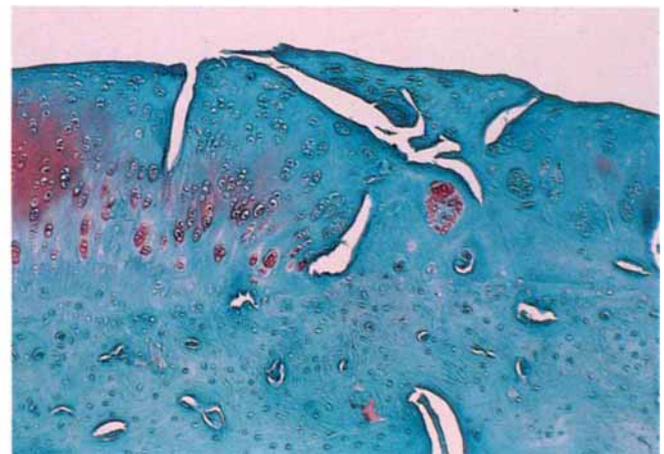
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Figure 2. Photomicrographs of lapine articular cartilage after staining with Safranin O. **A**, Longitudinal section of medial femoral cartilage from an unoperated rabbit. **B**, Medial femoral cartilage from a rabbit 6 weeks after partial medial meniscectomy (osteoarthritis [OA] control, prophylactic treatment study). **C**, Medial femoral cartilage from a rabbit that was subjected to partial medial meniscectomy and then treated with 0.25 ml/kg glycosaminoglycan-peptide association complex (GP-C) 3 times per week for 6 weeks (prophylactic treatment study). **D**, Medial femoral cartilage from a rabbit 20 weeks after partial medial meniscectomy (OA control, therapeutic treatment study). **E**, Medial femoral cartilage from a rabbit that was subjected to partial medial meniscectomy 20 weeks earlier and was allowed to develop an OA lesion over 6 weeks, before initiation of GP-C treatment (0.25 ml/kg 3 times per week for 14 weeks) (therapeutic treatment study). (Original magnification $\times 100$.)

The Mankin histologic scores (4) for all groups are shown in Figure 3A. There was a significant decrease in the Mankin scores even in the group that received

GP-C at the lowest dosage (0.05 ml/kg), compared with animals that had untreated OA (mean \pm SEM, 8.3 ± 0.2 versus 11.3 ± 0.5 ; $P < 0.01$). Furthermore, there

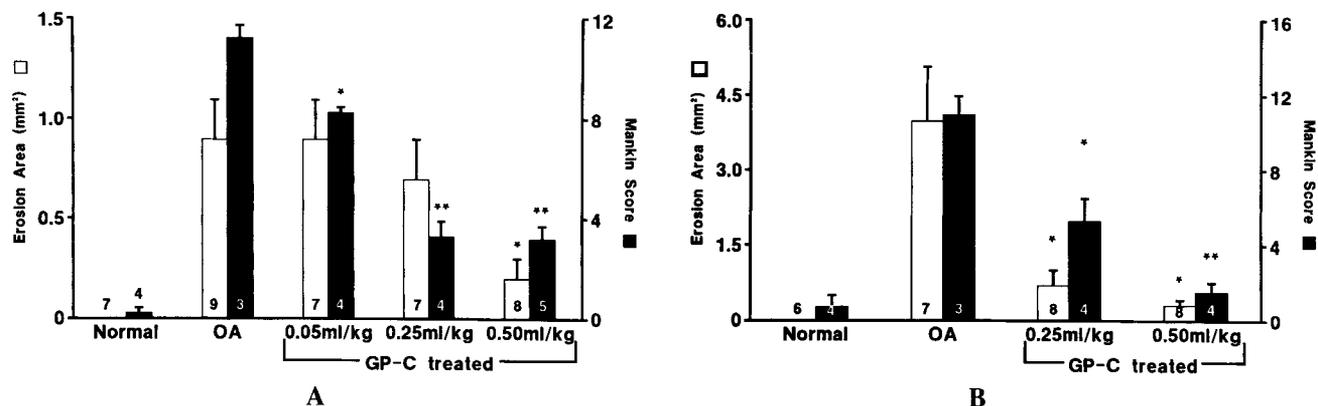


Figure 3. Erosion area and histologic grading of lapine articular cartilage. Erosion area was measured under a dissecting microscope at 10 \times magnification after staining with carbon black, as described by Meachim (22). Sections of medial femoral cartilage were fixed, embedded, sectioned, and stained, according to the method of Mankin et al (4). Grading of these specimens (Mankin score) was based on 4 separate subscores for structure, Safranin O staining, cellularity, and tidemark integrity. Values are the mean and SEM; the number of samples is shown at the base of each bar. * = $P < 0.01$ versus osteoarthritis (OA) controls; ** = $P < 0.001$ versus OA controls, by Student's 2-tailed t -test. **A**, Rabbits treated prophylactically with 0.05 ml/kg, 0.25 ml/kg, or 0.5 ml/kg glycosaminoglycan–peptide association complex (GP-C) 3 times per week for 6 weeks. **B**, Rabbits treated therapeutically with 0.25 ml/kg or 0.5 ml/kg GP-C 3 times per week from week 6 postsurgery until week 20.

was a decrease in Mankin score with increasing dosages of GP-C, suggesting a dose-dependent effect. As was seen with erosion area, however, Mankin scores in GP-C-treated OA groups remained higher than those in normal controls.

DNA and uronic acid levels in normal control and OA control cartilage were similar (Table 1). Hydroxyproline content, in contrast, was significantly decreased in OA cartilage compared with healthy cartilage ($P < 0.001$). Treatment with GP-C significantly increased tissue hydroxyproline levels. In the group treated with 0.5 ml/kg GP-C, hydroxyproline levels reached those found in normal cartilage.

Levels of both active and total neutral metalloproteinase were elevated in OA cartilage compared with normal control cartilage ($P < 0.001$), and enzyme in the OA cartilage was fully active (Figure 4A). Treatment with 0.05 ml/kg GP-C 3 times per week produced a significant reduction in active, but not total, enzyme. Dosage levels of 0.25 ml/kg and 0.5 ml/kg produced a further significant reduction in metalloproteinase levels.

TIMP levels in normal and OA cartilage were equivalent at 1.8 inhibitor units/gm (Figure 5). With GP-C treatment, TIMP levels rose in a dose-dependent manner. However, only in rabbits treated with 0.5 ml/kg GP-C was the TIMP level significantly higher than that found in OA or normal control cartilage.

Results of therapeutic treatment. Cartilage from unoperated control rabbits used in the second phase of

the study was smooth, white, and glistening, as described above. Cartilage from OA rabbits had a large central erosion on the medial femoral condyle, which stained deeply with carbon black (Figure 1D). The surface was pitted and dull, with marginal osteophytes. Treatment with either 0.25 ml/kg GP-C or 0.5 ml/kg GP-C 3 times per week from week 6 to week 20

Table 1. DNA, uronic acid, and hydroxyproline content of normal, osteoarthritic (OA), and glycosaminoglycan–peptide association complex (GP-C)-treated lapine cartilage*

Study group (n)	DNA	Uronic acid	Hydroxyproline
Prophylactic study			
Normal control (6)	0.6 \pm 0.06	11.5 \pm 1.3	21.7 \pm 1.0 [†]
OA control (6)	0.6 \pm 0.10	10.7 \pm 1.2	12.6 \pm 0.7
GP-C, 0.05 ml/kg (3)	0.7 \pm 0.07	10.7 \pm 0.6	15.9 \pm 0.5 [‡]
GP-C, 0.25 ml/kg (7)	0.6 \pm 0.05	10.5 \pm 1.0	15.0 \pm 0.8 [§]
GP-C, 0.5 ml/kg (5)	0.5 \pm 0.04	13.4 \pm 0.5	22.7 \pm 2.2 [†]
Therapeutic study			
Normal control (6)	0.8 \pm 0.04	11.9 \pm 0.7 [§]	20.4 \pm 1.1 [‡]
OA control (6)	0.8 \pm 0.04	9.2 \pm 0.9	14.7 \pm 1.1
GP-C, 0.25 ml/kg (5)	0.5 \pm 0.05 [¶]	9.3 \pm 0.8	17.3 \pm 1.4
GP-C, 0.5 ml/kg (6)	0.6 \pm 0.06 [§]	11.0 \pm 0.9	18.6 \pm 1.3 [§]

* DNA, uronic acid, and hydroxyproline contents ($\mu\text{g}/\text{mg}$ tissue) were determined after digestion of the tissue with proteinase K (27). DNA was assayed fluorimetrically (28), uronic acid colorimetrically (29), and hydroxyproline colorimetrically after hydrolysis with HCl (30,31). Values are the mean \pm SEM.

[†] $P < 0.001$ versus OA control group, by Student's 2-tailed t -test.

[‡] $P < 0.01$ versus OA control group, by Student's 2-tailed t -test.

[§] $P < 0.05$ versus OA control group, by Student's 2-tailed t -test.

[¶] $P < 0.003$ versus OA control group, by Student's 2-tailed t -test.

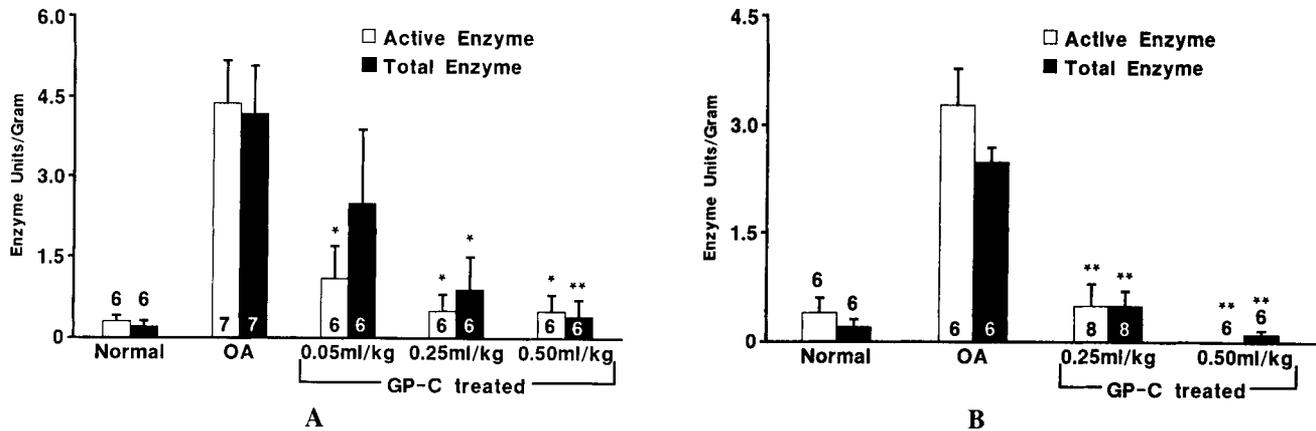


Figure 4. Levels of active and total metalloproteinase in lapine articular cartilage. Cartilage was extracted in buffered guanidine, centrifuged, and the supernatant dialyzed into assay buffer. Enzyme activity was determined on proteoglycan-containing polyacrylamide beads at neutral pH, in the presence or absence of APMA. Values are the mean and SEM enzyme units/gm wet weight cartilage; the number of samples is shown at the base of each bar. * = $P < 0.01$ versus osteoarthritis (OA) controls; ** = $P < 0.001$ versus OA controls, by Student's 2-tailed t -test. **A**, Rabbits treated prophylactically with 0.05 ml/kg, 0.25 ml/kg, or 0.5 ml/kg glycosaminoglycan-peptide association complex (GP-C) 3 times per week for 6 weeks. **B**, Rabbits treated therapeutically with 0.25 ml/kg or 0.5 ml/kg GP-C 3 times per week from week 6 postsurgery until week 20.

significantly reduced carbon black staining, as well as erosion area ($P < 0.01$) (Figures 1E and 3B).

Sections of medial femoral cartilage that had been prepared for histologic study are shown in Figure 2. Specimens from unoperated controls were normal and similar to those described above for the prophylactic study. OA cartilage showed significantly more pathologic changes at 20 weeks after surgery than had been seen at 6 weeks (Figure 2D). The cartilage surface was irregular, with fissures and clefts, and

exhibited prominent cell cloning, lack of Safranin O staining, loss of tidemark integrity, and general tissue disorganization.

Lesions that had formed during the 6-week period prior to the initiation of GP-C therapy showed signs of cartilage repair after treatment (Figure 2E). Surface irregularity was reduced and Safranin O staining was partially retained, as was tidemark integrity and cellularity. The Mankin scores for all groups are shown in Figure 3B. A significant decrease was observed in the group that received 0.25 ml/kg GP-C when compared with OA controls (mean \pm SEM, 5.3 ± 1.2 versus 11.0 ± 1.0 ; $P < 0.01$); further improvement was seen in animals given GP-C at a higher dosage.

Cartilage DNA content was again unchanged in OA compared with normal controls (Table 1). However, in contrast to the prophylactic study, GP-C treatment in the therapeutic study at both doses reduced tissue DNA content. Uronic acid and hydroxyproline levels were both decreased in OA cartilage, compared with cartilage from unoperated controls. Treatment with GP-C had no effect on cartilage uronic acid content, but GP-C treatment at the higher dosage (0.5 ml/kg) caused hydroxyproline content to approach normal levels.

Levels of both active and total neutral metalloproteinase were elevated in OA cartilage compared with normal control cartilage ($P < 0.001$), with enzyme in the OA cartilage again found to be fully active (Figure 4B). Treatment with 0.25 ml/kg GP-C pro-

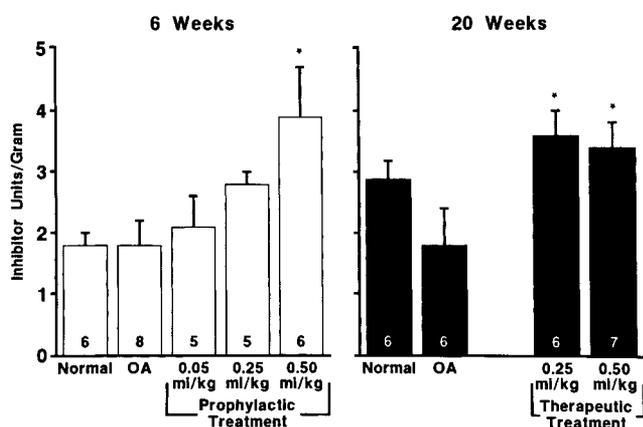


Figure 5. Levels of tissue inhibitor of metalloproteinase (TIMP) in lapine articular cartilage. Cartilage from normal, osteoarthritis (OA) control, or glycosaminoglycan-peptide association complex (GP-C)-treated rabbits was extracted as described in Figure 4 and the supernatants were assayed for TIMP, using active collagenase. Values are the mean and SEM inhibitor units/gm wet weight cartilage; the number of samples is shown at the base of each bar. * = $P < 0.05$ versus OA controls, by Student's 2-tailed t -test.

duced a significant reduction in both active and total forms of the enzyme ($P < 0.001$). When this dosage was doubled, metalloproteinase was virtually undetectable in the tissue.

TIMP was found to be slightly decreased in OA control cartilage compared with normal cartilage (Figure 5). With GP-C treatment, TIMP content increased to levels significantly higher than those found in OA cartilage ($P < 0.01$).

DISCUSSION

Current approaches to the management of osteoarthritis are far from ideal. The most potent therapeutic agents are either only partially effective or, as with corticosteroid derivatives, interfere with cartilage repair. In the 1960s, an agent that is a potent inhibitor of polymorphonuclear elastase, as well as a stimulator of proteoglycan and collagen synthesis (19,39), was developed in Switzerland, and subsequently marketed as Rumalon (GP-C). GP-C is manufactured from bovine cartilage and bone marrow and exists as an aqueous high molecular weight glycosaminoglycan-peptide complex containing amino acids and growth-stimulating peptides. Since its discovery, numerous clinical studies have demonstrated favorable effects of GP-C in the treatment of human OA (18).

In vitro studies have partially discerned how GP-C exerts its biologic effects on anabolism and catabolism. Astaldi et al observed that chick chondrocytes expressed their true phenotype in the presence of GP-C, while chondrocytes cultured without GP-C reverted to fibroblasts (33). Bollet found that 5% GP-C stimulated ^{35}S incorporation into PG by rat costal cartilage, as well as both normal and OA human articular cartilage, in culture (34). In contrast, Malemud and Sokoloff found that 1–2% GP-C failed to stimulate rabbit articular cartilage chondrocyte or skin fibroblast PG synthesis (35,36). In studies by Adam et al, collagen synthesis was stimulated by GP-C in human OA cartilage cultures, as determined by incorporation of ^3H -proline. Normal cartilage, in contrast, was not stimulated by the addition of GP-C (37,38). This preferential response of OA cartilage, but not normal cartilage, to GP-C was not seen in the other studies of PG synthesis mentioned above. Bassler et al have reported that human chondrocytes respond to GP-C, depending on its concentration in the medium, by producing increased amounts of PG, type II collagen, or DNA (39). In summary, it is clear that GP-C has the ability to stimulate matrix synthesis, but this is

greatly dependent on the conditions and tissues used in a particular study.

The effect of GP-C on cartilage catabolism has also been reported. Frei et al found that GP-C blocked autolysis of proteoglycans by endogenous enzymes in calf sternal cartilage, and concomitantly stimulated sulfur incorporation into proteoglycans (40). Others have tested GP-C for its ability to directly inhibit hyaluronidase, papain, and collagenase (41,42). It was concluded in those studies that one mechanism by which GP-C may exert its effects in OA is through inhibition of enzymes implicated in matrix breakdown. In contrast, our own unpublished studies have shown that GP-C by itself has little capacity to directly inhibit rabbit cartilage metalloproteinase.

The effect of GP-C in modifying the progression and extent of OA has also been reported. Neumuller et al (43) and Gialamas et al (44) showed that 0.5 ml/kg GP-C reduced the progression of early OA lesions in the rabbit meniscectomy model, but that advanced lesions did not respond to treatment. Barcelo and colleagues performed similar studies but used both qualitative and quantitative assessment techniques (45). Qualitatively, the cartilage had fewer surface changes, decreased loss of PG, and less disruption of the collagen after treatment with GP-C. By quantitative measures, cartilage thickness was maintained at near-normal levels by GP-C, but cell density remained similar to that found in untreated OA.

How GP-C mediates its effects on cartilage, or whether GP-C per se enters the cartilage, is entirely unknown. The major obstacle to answering this resides in the fact that GP-C is a tissue extract. To date, no single substance has been found in GP-C which displays the properties of the mixture itself. Pharmacokinetic studies have thus been precluded. In such situations, the generally accepted practice is to conduct pharmacodynamic studies to measure dose-dependent effects as a function of time (i.e., effect kinetics). This approach has been used in the studies reported here and in other investigations of GP-C.

The present studies have extended the observations of others by showing that GP-C not only blocks evolution of OA lesions treated prophylactically, but can also repair lesions formed prior to therapeutic treatment in a knee that remains destabilized. The results demonstrate that GP-C may accomplish this by reducing proteinase levels through selective blocking of proteinase synthesis, blocking the activation of latent metalloproteinase, or production of increased amounts of metalloproteinase inhibitor. At present, it is uncertain which mechanism is the most likely, or

whether all three occur simultaneously. In addition to these effects, GP-C increased the hydroxyproline content of the tissue, indicating that new matrix synthesis may be stimulated in response to GP-C treatment. Earlier studies by our group, using glycosaminoglycan polysulfuric acid ester (Arteparon; Luitpold Werke, Munich, FRG) in both rabbit and canine OA models, have shown similar effects on proteinase levels, but TIMP levels were not determined (14,46–48).

Stimulation of TIMP synthesis could be an important property of chondroprotective agents, since the endogenous mechanism for preventing cartilage degradation may be simply overwhelmed in OA. We have reported that TIMP is present in human articular cartilage (15) and suggested that a close balance exists between metalloproteinase and TIMP levels in normal cartilage (12). In OA, the proteinase levels rise proportionally higher than the levels of TIMP, leading to an excess of proteinase. This imbalance most likely results in excess matrix degradation. The beneficial effect of systemic TIMP administration in collagen-induced arthritis (49) lends further support to the view that TIMP may be very important in protecting cartilage from degradation. The ability of GP-C to raise TIMP levels in cartilage would provide a rational explanation for its ameliorative effect on OA.

In summary, the mechanism by which GP-C exerts its observed effects on OA is still uncertain. However, it is clear from this study that metalloproteinases and their endogenous inhibitor are favorably affected. Further studies of GP-C should be conducted to identify any growth factors it may contain, and also to determine whether GP-C can block endogenous production of interleukin-1.

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