Interleukin-1 Reversibly Inhibits the Synthesis of Biglycan and Decorin in Intact Articular Cartilage in Culture

HANS VON DEN HOFF, MARGRET DE KONING, JOS VAN KAMPEN, and JAN VAN DER KORST

ABSTRACT. Objective. To study the effect of interleukin-1 (IL-1) on the synthesis of proteoglycans biglycan (DSPG-I) and decorin (DSPG-II) in intact bovine articular cartilage.

Methods. Cartilage bearing sesamoid bones from the metacarpophalangeal joint were cultured with 10 ng/ml IL-1 for 2 days and labelled with [35S] sulfate. One sesamoid bone from each animal had been labelled ex vivo. The remaining 2 were cultured with IL-1 and allowed to recover in control medium before labelling. Control cultures were maintained in medium without IL-1 and labelled concurrently with the experimental series. The dermatan sulfate proteoglycans were purified from 4 M guanidinium chloride extracts of the cartilage by gel filtration on Sepharose CL-2B and CL-4B, on which they appeared as a single peak. Biglycan and decorin were separated by sodium dodecyl sulphide polyacrylamide gel electrophoresis in high salt. Individual lanes from the gel were cut in slices, which were dissolved and counted for radioactivity.

Results. Ex vivo, biglycan accounted for 4% and decorin for 2% of total incorporated sulfate. IL-1 reduced the synthesis of biglycan to 77% of the level of cultured controls and that of decorin to 73%. The synthesis of both proteoglycans returned to the control levels when the IL-1 was removed. IL-1 (10 ng/ml, 2 days) had no significant effect on total proteoglycan synthesis.

Conclusion. The inhibition of synthesis of biglycan and decorin by IL-1 might be important in the pathophysiology of cartilage destruction in rheumatic diseases. (J Rheumatol 1995;22:1520-6)

Key Indexing Terms:
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Two types of small, nonaggregating dermatan sulfate proteoglycans have been isolated from bovine articular cartilage. They are present in about the same molar amount as the large aggregating species. Their core proteins, although similar, are distinct gene products and both have a molecular mass of 45 kDa. They show 55% homology at the protein sequence level and have a central leucine rich region. Biglycan, or DSPG-I, generally carries 2 dermatan sulfate chains, while decorin, or DSPG-II, carries only one. The glycosaminoglycans (GAG) from bovine cartilage biglycan and decorin contain 25-29% and 40-44% idurionate, respectively. Decorin binds to collagen fibrils in the cartilage matrix. Decorin has also been shown to inhibit collagen fibril formation in vitro. It has, therefore, been suggested to be involved in the regulation of collagen fibril assembly. Decorin can also bind transforming growth factor-ß, and thereby modulate its activity. Biglycan is not associated with collagen in vivo, but is located in the vicinity of the chondrocyte. Little is known about the function of this proteoglycan. Similar proteoglycans, with varying iduronate contents, have been found in the extracellular matrix of various other connective tissues. They are also synthesized by several cell types in vitro. In general, dermatan sulfate proteoglycans seem to have a function in the regulation of cell adhesion and in intercellular communication.

In inflammatory and degenerative joint diseases, the extracellular matrix of cartilage undergoes degradation. The cytokine interleukin-1 (IL-1) has been suggested as one of the key mediators of cartilage destruction. In rabbit knee joints, exogenous IL-1 can induce the loss of proteoglycans and inhibit their synthesis. In vitro, IL-1 can induce the synthesis of matrix metalloproteinases. It can also stimulate the synthesis of plasminogen activator, while reducing the production of plasminogen activator inhibitor and tissue inhibitor of metalloproteinases. The expression of cartilage specific collagens is also inhibited by IL-1. The effects of IL-1 on the large aggregating proteoglycan, aggrecan, have been extensively studied in explant cultures. The synthesis of aggrecan and, to a lesser extent, that of the small proteoglycans is reduced. Aggrecan synthesized in the presence of IL-1 is of the same size and has the same GAG composition as control aggrecan. IL-1 also stimulates the release of aggrecan from the matrix.

To our knowledge, no previous studies on the specific effects of IL-1 on biglycan and decorin in articular cartilage...
have been published. We studied the effects of IL-1 on these proteoglycans in cultured anatomically intact cartilage.

**MATERIALS AND METHODS**

MCP joints of 6-month-old calves were obtained from the Amsterdam slaughterhouse. Nutrient mixture Ham F-12, 45% balanced salt solution/glucose (GBSS), and penicillin/streptomycin were from Gibco Europe (Paisley, Renfrewshire, UK). Human recombinant insulin-like growth factor I (IGF-I) was from Boehringer Mannheim GmbH (Mannheim, Germany) and human recombinant IL-1α was a gift from Hoffmann-La Roche (Nutley, NY, USA). Bovine serum albumin (BSA), fraction V globulin-free, was from Sigma (St. Louis, MO, USA) and sodium [35S] sulfate (400 mCi/mmol) from New England Nuclear (Boston, MA, USA). Instagel II liquid scintillation fluid was from Packard (Downers Grove, IL, USA). The guanidinium chloride (GuHCl) from Sigma was pretreated with activated charcoal.

Cartilage cultures. Both medial sesamoid bones were dissected from the MCP joints of each forefoot of 6-month-old calves under aseptic conditions. The adhering soft tissue was carefully removed. Eight groups, each consisting of the 4 sesamoid bones from a single animal, were obtained in this way. Cartilage from the sesamoid bones of any individual calf is uniform with respect to GAG synthesis, GAG content, and proteoglycan composition. The groups were paired and each pair was cultured and processed simultaneously, one as a control and the other as an experimental series. The sesamoid bones were cultured in either 20 ml control medium or 20 ml control medium supplemented with 10 ng/ml IL-1. The control medium consisted of Ham F-12 containing 500 μM SO₄, 0.1% penicillin/streptomycin, 0.1% BSA, and 5 ng/ml IGF-I. The sesamoid bones were incubated in 50 ml polypropylene tubes at 37°C in a humidified atmosphere of 5% CO₂ in air. Media were changed every other day. The cultures were labelled for 17 h with 40 μCi/ml of [35S]SO₄ in the appropriate medium. Less than 1% of the macromolecular [35S]SO₄ is released into the culture medium during labelling. The sesamoid bones were then washed in cold GBSS at 4°C, and the cartilage was removed in 1–2 mm² pieces and washed again 3 times in cold GBSS at 4°C. This procedure reduced the amount of free label in the cartilage to less than 1% of that of total incorporated label. The first sesamoid bone from every series was labelled *ex vivo* in control medium. The second bone of an experimental series was cultured with 10 ng/ml IL-1 for 2 days and labelled. The second sesamoid bones were also cultured with IL-1 for 2 days, but were then changed to control medium and labelled at Days 4 and 9. The sesamoid bones from a control series were cultured in control medium for up to 9 days and labelled concurrently.

**Extraction and gel filtration.** The cartilage samples were cut into 20 μm slices in a cryostat. The slices were lyophilized, weighed, and extracted for 60 h at 4°C in 4 M GuHCl/potassium acetate buffer (pH 5.8) in the presence of proteinase inhibitors. The buffer contained 10 mM EDTA, 5 mM benzamidine HCl, 100 mM 6-aminoacaproic acid, 10 mM PMSF, and 5 mM N-ethylmaleimide. The residue was dissolved in 0.5 M NaOH at 60°C. Typical extraction percentages were 95% for total GAG and 83% for labelled GAG. Extracts were applied to Sepharose CL-2B columns (1 × 196 cm) and eluted at 3.0 ml/h with a dissociative buffer of pH 6.1, which contained 4 M GuHCl, 0.1 M sodium sulfate, and 0.05 M sodium acetate. Samples derived from the same calf were run on the same column. The fractions (2 ml each) containing the small proteoglycans were pooled, concentrated, and applied to a Sepharose CL-4B column (0.66 × 140 cm). The flow rate was 2.7 ml/h and 0.7 ml fractions were collected. The GAG content of the fractions was determined by the DMB method and their radioactivity by liquid scintillation counting. The recovery from both Sepharose CL-2B and CL-4B was more than 98%. The purified samples were concentrated, precipitated with ethanol, and dried.

**Analysis of GAG.** In a separate experiment the small proteoglycans were pooled after Sepharose CL-2B chromatography and then digested with proteinase K. The proteinase was inactivated for 10 min at 95°C, and GAG were sequentially digested with 0.5 U/ml of chondroitin AC lyase, of chondroitin ABC lyase, and of endo-β-galactosidase, each for 18 h at 37°C. After each step a sample of the digest was applied to a Sepharose CL-6B column. The decrease of the peak area of the GAG on CL-6B was used to estimate the extent of digestion. Sequential digestion showed that GAG of the peak of labelled small proteoglycans on Sepharose CL-2B contained 74% D-glucuronic acid residues, 11% L-iduronic acid residues, and 16% keratan sulfate. The presence of a large amount of keratan sulfate indicates that this peak still contained some large proteoglycans and possibly fibromodulin. The samples from Sepharose CL-2B were therefore, run on Sepharose CL-4B.

SDS-PAGE. The material from Sepharose CL-4B was dissolved in sample buffer containing 0.08 M Tris/HCl, 1% SDS, 0.01% bromophenol blue, 16% glycerol, 6 M urea and 5% β-mercaptoethanol, pH 6.8. Rainbow markers were diluted 1:1 in sample buffer. The samples were heated to 60°C for 2 h and applied to a 4–15% acrylamide/DATD gel in 0.375 M Tris with a 3.3% stacking gel. The 8 samples from an experimental series and its control series were analyzed on the same gel. The gels (12 × 14 × 0.3 cm) were run at 20 mA overnight in electrophoresis buffer containing 0.05 M Tris/HCl, 0.38 M glycine, 0.1% SDS, and 0.007% β-mercaptoethanol, pH 8.4. After fixation, the gels were stained with alcian blue30, destained, and scanned with an LKB laser scanner. Individual lanes were cut from the gel, frozen in dry ice, and sliced with a Mickle gel slicer. The slices (1 mm) were dissolved in 250 μl 2% periodic acid at 60°C and counted for radioactivity in Instagel. Chondroitin sulfate standards were also run on a gel and scanned. The amount of small proteoglycans in the samples was determined after integration of the scans. The apparent molecular mass of biglycan and decorin was estimated from linear plots of the logarithm of the molecular mass of protein standards against their relative mobility (Rf) in the gel.

**Hydrophobic interaction chromatography.** Some purified samples from Sepharose CL-4B were also run on an Octyl-Sepharose column (0.7 × 4 cm) as described, to verify the separation of biglycan and decorin in gel electrophoresis. The samples were applied in 4 M GuHCl, 0.15 M sodium acetate, pH 6.3, and allowed to bind for 2 h. The column was washed with 3 volumes of the same buffer, and the samples were eluted with a gradient of 0–1% CHAPS. Comparison of the 2 separation methods showed that less than 7% of the biglycan migrated to the lower (decorin) band in gel electrophoresis.

**Calculations.** The total GAG synthesis rate was determined from the total amount of macromolecular [35S] sulfate in the 4 M GuHCl extract and the NaOH digest of the residue. The synthesis of biglycan and decorin was calculated from the total GAG synthesis rate, the amount pooled on Sepharose CL-2B and CL-4B, and
the radioactivity associated with the peaks on the gel. The significance of differences between group means was calculated by 2-way analysis of variance and the analysis of simple effects. All results are expressed as mean ± SEM with N = 4 unless stated otherwise.

RESULTS

Effect of IL-1 on total GAG synthesis and content. Four cartilage bearing sesamoid bones from the same animal were cultured for 1, 2, 4, and 9 days and labelled with [35S] sulfate. The average ex vivo (Day 1) GAG synthesis rate was 1.95 ± 0.15 nmol SO4/mg dw/day (N = 8). The GAG synthesis rates on the following days were expressed as percentages of the individual ex vivo rates to compensate for the variation between animals (Figure 1). The GAG synthesis rate of the controls was found to have dropped to 61.6 ± 7.2% of the ex vivo rate, on Day 2, after which it increased. The decrease probably represents the adaptation to the culture conditions. In the experimental series, an IL-1 concentration of 10 ng/ml (for 2 days) was chosen because it does not enhance proteoglycan degradation in this culture system. IL-1 reduced the synthesis rate to 48.2 ± 5.1% by Day 2, but the difference with the control was not significant. This slight difference persisted to Days 4 and 9. Figure 1b shows the total GAG content of the cartilage during culture. The average ex vivo GAG content was 171.5 ± 4.8 μg GAG/mg dw (N = 8). No significant changes in GAG content occurred during the culture period.

Purification of the dermatan sulfate proteoglycans. Cartilage samples from the above experiments were extracted with 4 M GuHCl, and the extracts run on Sepharose CL-2B columns (Figure 2). The proteoglycans eluted in 2 separate peaks, one with a Kav of 0.3 and one with a Kav of 0.7. The first peak represents the large aggregating proteoglycan (aggrecan); the second peak, smaller proteoglycans. Sequential digestions showed that the latter was enriched in dermatan sulfate. The peak containing the dermatan sulfate proteoglycans, was pooled as indicated, concentrated, and applied to a Sepharose CL-4B column for further purification (Figure 3). The small proteoglycans eluted as a single peak at Kav 0.45, with the labelled molecules slightly larger than the non-labelled molecules. A considerable amount of mainly non-labelled aggrecan eluted in front of the small proteoglycans. The small proteoglycans were pooled in such a way that all labelled molecules were included. This pool was prepared for electrophoresis and run on an SDS-polyacrylamide gradient gel.

SDS-PAGE. In Figure 4 a typical gel profile of a purified sample is compared with profiles of biglycan and decorin standards. The Rf values of the nonlabelled standards were
fraction no.

Fig. 3. Sepharose CL-4B chromatography of small proteoglycans. The small proteoglycans pooled from Sepharose CL-2B (Figure 2) were concentrated and run on Sepharose CL-4B columns. Radioactivity (—) and chondroitin sulfate (CS) (---) was measured in the fractions. The small proteoglycans were pooled as indicated by the vertical lines. Void volume and total volume are indicated by the arrows.

Fig. 4. SDS-PAGE of a purified sample and biglycan/decorin standards. The small proteoglycans pooled from Sepharose CL-4B (Figure 3) were run on a 4–15% polyacrylamide gradient gel in 0.375 M Tris (a). Separate lanes were scanned at 633 nm (•—•) and sliced. The radioactivity of the slices was determined using scintillation counting (•—•). The profiles of standard preparations of biglycan (mature bovine cartilage) and decorin (fetal bovine skin) are also shown (b). The position of molecular weight markers (kDa) is indicated by the arrows.

Effect of IL-1 on the synthesis of biglycan and decorin. Gel profiles of labelled small proteoglycans of a typical series of samples are shown in Figure 5. Only the upper 75% of the gel is shown \( R_\text{f} = 0-0.75 \). Smaller \( ^{35} \text{S} \)-labelled molecules were not detected in the lower part of the gel. The \textit{ex vivo} profiles of samples from all the different animals were similar (Figure 5a). The synthesis of both biglycan and decorin in the controls decreased in culture. Treatment with IL-1 reduced the synthesis of both proteoglycans to below the corresponding levels in the controls (Figure 5b). Two days after the cytokine was removed the synthesis of both proteoglycans was still reduced (Figure 5c). By the 7th day of recovery, the synthesis of biglycan and decorin had returned to control levels (Figure 5d).

Synthesis rates of biglycan and decorin were calculated from total synthesis rates (Figure 1), pooled fractions after gel filtration (Figures 2 and 3) and radioactivity in the respective peaks (Figure 5). The \textit{ex vivo} synthesis rates of biglycan and decorin were 0.075 ± 0.007 and 0.039 ± 0.004 nmol SO\(_4\)/mg dw/day (\( N = 8 \)). Biglycan and decorin thus accounted for 4 and 2% of the total \textit{ex vivo} synthesis rate. The synthesis rates for the following days are given as percentages of the individual \textit{ex vivo} value (Figure 6). This was done to compensate for the variation in \textit{ex vivo} synthesis rates among the samples from different animals. The synthesis of biglycan in control cultures decreased with time (Figure 6a). On Day 2, the synthesis rate of biglycan was 83.5 ± 5.0% of the \textit{ex vivo} rate. After culture with IL-1, the biglycan synthesis rate was significantly lower than that in the control; 64.1 ± 7.4% (\( p < 0.01 \)). The difference between control and IL-1 treated samples persisted to Day 4, but by Day 9 the synthesis of biglycan had returned to control levels. The synthesis of decorin followed much the same course (Figure 6b). In control cultures the synthesis of decorin decreased to 73.2 ± 2.7% of the \textit{ex vivo} synthesis rate. Culturing with IL-1 reduced decorin synthesis to 53.4 ± 3.0% by Day 2 (\( p < 0.01 \)). This difference also persisted to Day 4 but by Day 9 the synthesis of decorin had also returned to control levels. The amount of nonlabelled biglycan and decorin in the gels.
Fig. 5. SDS-PAGE of purified samples from an experimental series and a control series. Samples from cartilage labelled on Day 1 (a), 2 (b), 4 (c), and 9 (d) were extracted, purified and analyzed on SDS-polyacrylamide gels. The profiles of experimental samples (•••••) and corresponding controls (-----) are shown. Only the upper 75% of the gels is shown. The vertical lines indicate the peak areas for biglycan (B) and decorin (D).

Fig. 6. The effect of IL-1 on the synthesis of biglycan and decorin. The areas of the peaks of biglycan and decorin on gel (Figure 5) were used to calculate their respective synthesis rates (see Materials and Methods). The results are shown as percentage of the Day 1 level for control (hatched bars) and experimental series (open bars). Panel a shows the synthesis of biglycan; Panel b, that of decorin. The significance of differences between group means was tested by 2-way ANOVA and the analysis of simple effects. Significant differences between treated groups and the corresponding controls are indicated by the asterisks (*** = p < 0.01).

was determined using standards of chondroitin sulfate. The amounts of small proteoglycans in the cartilage were 11.0 ± 0.4 and 3.7 ± 0.1 µg GAG/mg dw for biglycan and decorin, respectively (mean ± SEM, N = 32). No significant changes were observed during culture.

DISCUSSION

The separation of biglycan and decorin by SDS-PAGE at high ionic strength is thought to occur through the self-association of biglycan. However, biglycan can occur with one instead of 2 dermatan sulfate chains, and this might interfere with its self-association. We therefore verified the separation of some samples by hydrophobic interaction chromatography, which depends on the properties of the core protein. The apparent molecular masses that we found for nonlabelled biglycan and decorin correlate well with those obtained by
It is possible to restore their synthesis by loading the proteoglycans in bovine articular cartilage during culture. It is probably caused by the synthesis of others working with immature bovine cartilage. Similar changes, caused by a decrease in GAG chain length, occur in human articular cartilage. The labelled biglycan and decorin in our samples were larger than the nonlabelled species, a phenomenon also observed in samples from human cartilage. It is probably caused by the synthesis of longer GAG chains in culture. The synthesis of both biglycan and decorin in the controls decreased during culture. This extends our previous results, which show that there is a decrease in the synthesis of small proteoglycans in bovine articular cartilage during culture. It is possible to restore their synthesis by loading the cartilage. The decrease seems, then, to be related to the absence of stress on the cartilage during normal culture. Our present results show that incubation with IL-1 reduced the synthesis of both biglycan and decorin to below the corresponding levels in cultured controls. The synthesis of both species returned to control levels following the removal of cytokine. The regulation of synthesis of dermatan sulfate proteoglycans has also been studied in other systems. IL-1 increases considerably the mRNA level of the dermatan sulfate proteoglycan in fibroblasts although the synthesis is only slightly enhanced. In cultures of bovine chondrocytes retinoic acid inhibited the synthesis of biglycan, while decorin synthesis underwent a 12 to 18-fold increase. The mRNA levels underwent similar changes. In fibroblast cultures transforming growth factor-β increased the expression of biglycan while that of decorin remained relatively unaffected. These reports demonstrate that there is a difference in the regulation of biglycan synthesis and that of decorin synthesis. Our results show that, in intact articular cartilage, the regulation of synthesis of biglycan and decorin by IL-1 is similar.

After 2 days of culture with IL-1, the total amount of GAG in the matrix was unchanged, and there were no changes in biglycan and decorin content. In cultures of explanted cartilage a considerable loss of proteoglycans occurs within 3 days. Continuous infusion of IL-1 in rabbit knee joints for 14 days resulted in a 50% reduction in the amount of intact decorin core protein. Both decreased biosynthesis and enhanced degradation contribute to the depletion of small proteoglycans in articular cartilage in response to IL-1.

Decorin is thought to be involved in the regulation of collagen fibril assembly, and in the stabilization of the collagen network. The inhibition of synthesis of decorin by IL-1 might, therefore, be an important process in the pathophysiology of cartilage destruction in rheumatic diseases. A further function of the small proteoglycans might lie in the regulation of growth factor activity. A reduction in the content of these proteoglycans might disturb the balance of growth factors in diseased tissue. Biglycan probably has other specialized functions because its localization and regulation of synthesis seem to be different.

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