Inhibitors of Hyaluronan Export Prevent Proteoglycan Loss from Osteoarthritic Cartilage

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ABSTRACT. ***Objective.*** Osteoarthritis (OA) is characterized by cartilage erosion, proteolysis of aggrecan and collagen, and disturbed synthesis rates of aggrecan and hyaluronan by chondrocytes. The hypothesis is tested that hyaluronan overproduction contributes to aggrecan loss from osteoarthritic cartilage.

Methods. Human chondrocytes or bovine cartilage explants were incubated with interleukin 1ß (IL-1ß) to induce upregulation of hyaluronan and downregulation of aggrecan. OA was induced by injection of iodoacetate into the synovial cavity in rat knees. Hyaluronan export was inhibited by ATP-binding cassette transporter inhibitors such as the multidrug resistance (MDR) inhibitors valspodar or verapamil. The concentration of aggrecan was measured in cell culture media or visualized histochemically in cartilage tissue sections.

Results. Valspodar inhibited hyaluronan export from human chondrocytes in cell culture selectively without reducing aggrecan secretion. Valspodar and other MDR inhibitors prevented loss of aggrecan from osteoarthritic cartilage explants in culture. Verapamil prevented loss of aggrecan from cartilage in osteoarthritic rat knees.

Conclusion. Hyaluronan is synthesized at plasma membranes and exported out of the cell. We recently identified an ATP-binding cassette transport system that is responsible for hyaluronan export. A number of ATP-binding cassette transport inhibitors are known and are in use clinically. These inhibitors were used here to inhibit hyaluronan export and to prevent aggrecan loss from arthritic cartilage. New drugs for treatment of arthritis are suggested by these studies. (J Rheumatol 2005;32:690–6)

Key Indexing Terms:
HYALURONAN EXPORT INHIBITORS CARTILAGE OSTEOARTHRITIS AGGRECAN

Osteoarthritis (OA) is accompanied by a loss of cartilage at the joint surface. The cartilage goes through different stages during pathogenesis[1]. In the early stage, chondrocytes replace the loss of cartilage by increased proliferation matrix synthesis. Simultaneously, lacunae of edema and increased water-binding occur. Increased water content leads to softening of the cartilage matrix. These phenomena are observed before fibrillation or cartilage erosion. At the second stage new cartilage production cannot compensate for the loss, and at the third stage loss of cartilage is complete. It is known from other injuries that increased hyaluronan production can be a cause of edema and water accumulation[2-4].

The cartilage matrix consists of 2 main components: type II collagen and high molecular weight aggrecan. Aggrecan accumulates on a backbone of hyaluronan that may be anchored in the plasma membrane of chondrocytes at the hyaluronan synthase site and further bound by the cell surface receptor CD44[5]. The chondrocytes are responsible for synthesizing and degrading the matrix. Biosyntheses of hyaluronan and proteoglycans (PG) have different mechanisms and occur in different compartments: PG are synthesized in the Golgi and exocytosed by vesicles. Hyaluronan is polymerized at the inner surface of plasma membranes[6] and exported by ATP-binding cassette (ABC) transporters[7,8]. Both components aggregate in the extracellular matrix[9,10].

In healthy cartilage, matrix components are synthesized and degraded at similar rates. In osteoarthritic cartilage the matrix appears more swollen and amorphous[11]. Hyaluronan production is increased compared to PG[12], and enhanced synthesis of proteases, including collagenases, causes dissolution of collagen fibrils[13]. In cell and organ cultures, cellular mediators such as interleukin 1ß (IL-1ß) can induce similar reactions[14-19]. Increased hyaluronan synthesis precedes the stimulation of protease synthesis[20,21].

Previously it was thought that proteolytic degradation of collagen and aggrecan was primarily responsible for cartilage breakdown. Efforts to develop protease inhibitors led to compounds that were chondroprotective *in vitro* and in animal models, but results from clinical trials were equivocal[22,23].

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This study was undertaken to investigate the hypothesis that overproduction of hyaluronan in osteoarthritic cartilage contributes to disintegration. A test of this hypothesis has become feasible after the discovery of drugs that selectively inhibit hyaluronan, but not PG synthesis. Recently, we reported that a variety of multidrug resistance (MDR) inhibitors interfere with hyaluronan export from human cells[8]. These drugs inhibit ABC transporters and belong to the class of inhibitors of MDR proteins and MDR associated proteins. This study examined their effect on chondrocyte cell cultures and cartilage organ cultures, and evaluated their effect on PG loss in an animal model of OA.

MATERIALS AND METHODS

Materials and cells. Valspodar was a kind gift from Novartis, Basel, Switzerland, and other chemicals were from Sigma Chemical Co., Deisenhofen, Germany. Bovine articular cartilage was from a local slaughterhouse. A temperature-sensitive human chondrocyte cell line (tsT/AC62) was provided by Dr. M. Goldring, Boston, MA[24].

Determination of hyaluronan concentration. The cells were incubated for 2 days at 37°C and aliquots (5 and 20 µl) of the culture medium were used for measurement of the hyaluronan concentration in the cell culture medium by an ELISA[25]. Briefly, the wells of a 96 well Covalink-NH-microtiter plate (Nunc) were coated with 100 µl of a mixture of 100 mg/ml hyaluronan (Healon®), 9.2 µg/ml of N-hydroxysuccinimide-3-sulfonic acid, and 615 µl/ml of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide for 2 h at room temperature and overnight at 4°C. The wells were washed 3 times with a solution of 2 M NaCl, 41 mM $MgSO_4$, 0.05% Tween-20 in 50 mM phosphate buffered saline (PBS), pH 7.2 (buffer A), and once with 2 M NaCl, 41 mM $MgSO_4$, in PBS, pH 7.2. Additional binding sites were blocked by incubation with 300 µl of 0.5% bovine serum albumin (BSA) in PBS for 30 min at 37°C. Calibration of the assay was performed with standard concentrations of hyaluronan ranging from 15 ng/ml to 6000 ng/ml in equal volumes of culture medium, as used for measurement of the cellular supernatants. A solution (50 µl) of the biotinylated hyaluronan binding fragment of aggrecan (Applied Bioligands, Winnipeg, MB, Canada) in 1.5 M NaCl, 0.3 M guanidinium hydrochloride, 0.08% BSA, 0.02% NaN_3, 25 mM phosphate buffer, pH 7.0, was preincubated with 50 µl of the standard hyaluronan solutions or cellular supernatants for 1 h at 37°C. The mixtures were transferred to the hyaluronan-coated test plate and incubated 1 h at 37°C. The microtiter plate was washed 3 times with buffer A and incubated with 100 µl/well of a solution of streptavidin-horseradish-peroxidase conjugate (Amersham) at a dilution of 1:100 in PBS, 0.1% Tween-20 for 30 min at room temperature. The plate was washed 5 times with buffer A and the color was developed by incubation with a 100 µl/well of a solution of 5 mg O-phenylenediamine and 5 µl 30% H_2O_2 in 10 ml of 0.1 M citrate-phosphate buffer, pH 5.3, for 25 min at room temperature. The adsorption was read at 490 nm in an ELISA reader. The concentrations in the samples were calculated from a logarithmic regression curve of the hyaluronan standard solutions.

Inhibition of hyaluronan synthesis by valspodar in human chondrocytes. The human chondrocyte cell line (tsT/AC62) was grown in RPMI media at 32°C to near confluency and harvested by trypsinization as described[24]. The cells were suspended in an alginate solution (1.2% in 0.9% NaCl) at a cell density of 4×10^6 cells/ml and pressed though a 22G syringe dropwise (3 drops) into a sterile solution of 102 mM $CaCl_2$ that had been added in the wells of a microtiter plate. This treatment leads to the formation of alginate beads containing chondrocytes. The beads in the wells of the microtiter plate were washed with PBS and incubated with RPMI media for 5 days at 39°C. The media were then replaced with fresh media with and without IL-1ß (200 pg/ml) and increasing concentrations of valspodar and incubated for another 3 days at 39°C. The beads were washed with PBS and solubilized with a solution of 125 µl 55 mM Na citrate in 0.9% NaCl, pH 6.05, for 10 min at 37°C. The cells were sedimented at 2000 g for 5 min and the supernatants were supplemented with 12.5 µl of 200 µg/ml papain in 0.1 M Na acetate, pH 5.53, 50 mM EDTA, 0.9% NaCl, 5 mM cysteine and also incubated for 20 h at 37°C. Aliquots were taken for determination of the hyaluronan concentration by ELISA.

Effect of valspodar on PG synthesis in human chondrocytes. Human chondrocytes were grown as described above. Aliquots were taken for determination of the PG concentration using a color reaction as described[26]. For measurement of PG synthesis rate, the chondrocytes in the wells of the microtiter plates were supplemented with 12.5 µl $[^{35}S]SO4^{2-}$ (0.5 mCi/ml) 20 h before harvest. Aliquots (20 µl) were used for the determination of radioactivity incorporated into $[^{35}S]$PG as described[27].

Determination of the inhibitory effects of drugs on PG loss from osteoarthritic bovine cartilage. A bovine knee was obtained from a local slaughterhouse and slices of cartilage (0.5 cm^2) were incubated in 1 ml of RPMI media containing 10% fetal bovine serum in the presence and absence of IL-1ß (5 ng/ml) and different concentrations of MDR inhibitors for 3 days at 37°C. The tissues were fixed in 4% phosphate buffered formalin and embedded in paraffin. Sections of 6 µm were cut and stained histologically with fast green and safranin O. This stains proteoglycans red and other material greyish green.

Treatment of osteoarthritic rats with verapamil. Male Wistar rats (Charles River, Sulzfeld, Germany) having a body weight of 150 g were fed on ordinary laboratory diet and were housed in cages with a floor area of 800 cm^2. Light-dark cycles were maintained at 12 h/12 h. Room temperature was 21°C. All procedures were approved by the district veterinary administration of Muenster and complied with the Animal Protection Act of Germany. The operations were performed under anesthesia with isofluran/N_2O. Osteoarthritic damage was induced in the left knees of 6 Wistar rats by injection of a solution of 50 µl of 100 mM iodoacetate into the synovial cavity. The right knees received 50 µl of saline and served as controls. Three rats were fed with normal drinking water and 3 with drinking water containing 0.75 mg/ml verapamil, a concentration used previously[28]. After 17 days, the rats were sacrificed and the articular cartilage was analyzed by histology. The knees were fixed in 4% phosphate buffered formalin, decalcified in D-Calcifer (Shandon, Life Sciences International GmbG, Frankfurt, Germany), and embedded in paraffin. Longitudinal sections 6 µm thick were cut through the long axis of the tibia in the sagittal plane. Acid polysaccharides were stained with alcian blue and the preparation was counterstained with nuclear fast red.

RESULTS

Valspodar inhibits hyaluronan synthesis in human chondrocytes. The action of the MDR inhibitor valspodar on hyaluronan synthesis was first investigated in a human chondrocyte culture that is immortalized with a retrovirus expressing a temperature-sensitive mutant of SV40-large T antigen. The cells proliferate at 32°C, and at 39°C they mimic the adult articular chondrocyte phenotype, particularly in alginate culture, and show characteristic responses to IL-1 in a manner similar to chondrocytes obtained from biopsy[24]. They were cultured for 5 days in alginate beads and then incubated with IL-1ß in the presence of increasing concentrations of valspodar for 3 days. The concentrations of hyaluronan and PG were determined in the media, the alginate, and the cells. $[^{35}S]$Sulfate incorporation into PG was determined in a parallel series of experiments during the last 24 h of incubation.

Interleukin treatment caused an increase of hyaluronan

production in alginate in accord with previous results[16] (Figure 1). Valspodar almost reversed this increase of hyaluronan concentration in the alginate beads to levels comparable to interleukin-free cultures. Valspodar also reduced the amount of hyaluronan bound to chondrocytes (Figure 1). However, there were no detectable differences in the concentration of hyaluronan in the culture medium in the interleukin and valspodar treated and untreated samples. Valspodar also reduced the amount of hyaluronan on chondrocytes and in alginate when cells were not stimulated with interleukin (data not shown).

The effect of interleukin treatment on PG metabolism is shown in Figure 2. It did not significantly change the amount of PG in the alginate beads, but decreased the synthesis rate, in agreement with earlier results[16]. Valspodar did not significantly change the PG synthesis rate and its concentration in alginate. Similar results were obtained with other hyaluronan export inhibitors such as benzbromarone or trequinsin on bovine chondrocyte cultures. Thus hyaluronan export inhibitors selectively inhibited only hyaluronan synthesis, and not general PG synthesis and deposition in the alginate matrix. However, valspodar decreased the amount of PG on chondrocytes (Figure 2).

Valspodar inhibits PG loss from bovine cartilage. If overproduction of hyaluronan contributed to PG loss from osteoarthritic cartilage, inhibition of hyaluronan synthesis should normalize it. Slices of bovine cartilage were incubated with interleukin and with 2 concentrations of valspodar. After 5 days the samples were fixed and stained with safranin-O for PG. Figure 3 shows that valspodar inhibited PG loss in a dose-dependent manner, from 3 to 12 µM.

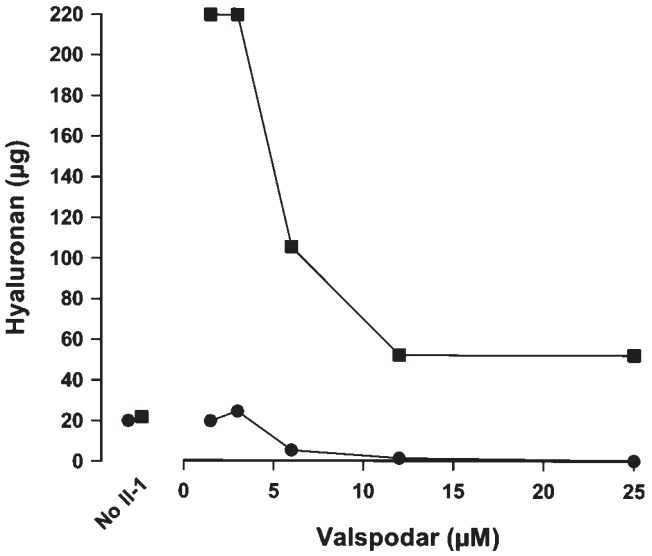

Figure 1. Concentration-dependent inhibition of hyaluronan deposition of human chondrocytes by the MDR inhibitor valspodar. The human chondrocyte cell line tsT/AC62 was cultured in alginate beads without and with 5 ng/ml IL-1ß and increasing concentrations of valspodar. The amount of hyaluronan in alginate (■) and on the cells (●) was determined. Values represent SD of duplicate measurements (n = 2). (Similar results were obtained in another independent experiment.)

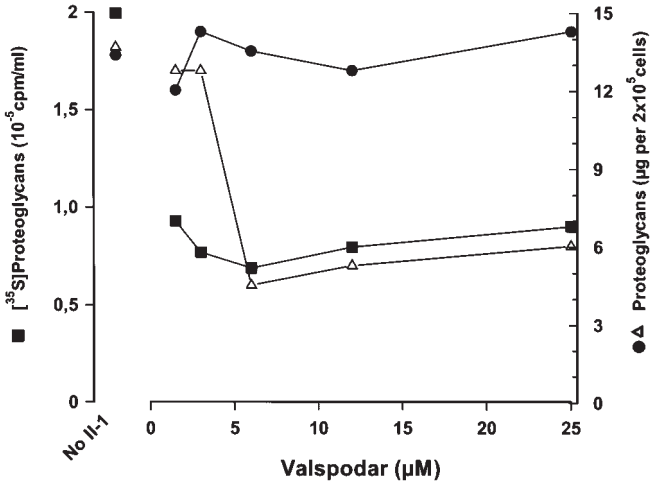

Figure 2. Effect of increasing concentration of valspodar on PG synthesis and deposition by human chondrocytes. The human chondrocyte cell line tsT/AC62 was cultured in alginate beads without and with 5 ng/ml IL-1ß and increasing concentrations of the MDR inhibitor valspodar. The amount of proteoglycans in alginate (●) and on the cells (Δ) was determined. The rate of PG synthesis was measured by incubation with [^{35}S]sulfate and determination of radioactivity incorporated into [^{35}S]proteoglycans in alginate (■). Values represent SD of duplicate measurements (n = 2). (Similar results were obtained in another independent experiment.)

Specificity of inhibitors. The ABC transporter inhibitors verapamil, nicardipine, nimodipine, and bepridil were evaluated for their protective effect on PG loss from bovine cartilage. Figure 4 shows that verapamil gave a protection similar to that of valspodar. Also, the verapamil analogs nicardipine and nimodipine showed fairly good protection, whereas bepridil (a nonselective Ca^{2+} channel blocker) was ineffective at the same concentration.

Inhibition of PG loss by verapamil in a rat model of OA. The experiments described above indicated that both valspodar and verapamil protected osteoarthritic cartilage from PG loss. Because verapamil is an approved drug for treatment of cardiac arrhythmias and effective concentrations have been determined in rats[28], its protective capacity was evaluated in a rat model of OA[29]. Osteoarthritic damage was induced in the left knees by injection of iodoacetate into the synovial cavity. The right knees received saline and served as controls. Three rats were fed with normal drinking water and 3 with drinking water containing verapamil. After 17 days the rats were sacrificed and the articular cartilage was analyzed by histology. Acid polysaccharides were stained with alcian blue and the preparation was counterstained with nuclear fast red. Samples were taken from each knee joint and 3 slices of each joint were stained, yielding similar images. Figure 5 shows that verapamil inhibited PG loss.

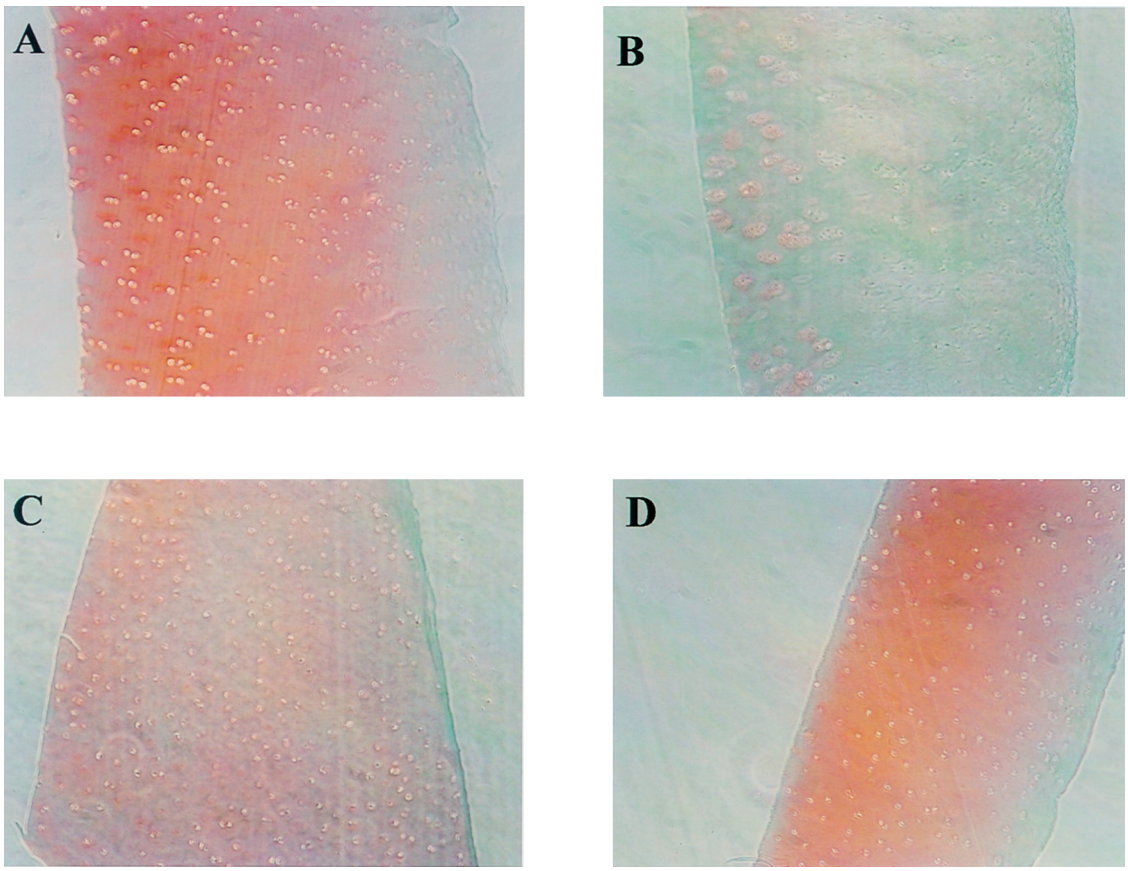

Figure 3. Histologic analyses of bovine articular cartilage cultured with and without valspodar. (A) control, (B) IL-1ß, (C) IL-1ß + 3 µM valspodar, (D) IL-1ß + 12 µM valspodar. PG were stained in thin sections with safranin-O and counterstained with fast green.

DISCUSSION

Previously we described a variety of inhibitors for hyaluronan export from human cells[8]. They belong to the class of MDR and MDR protein inhibitors. Two of these inhibitors, valspodar and verapamil, were evaluated on human chondrocyte cell cultures, on bovine cartilage, and in a rat model of OA. For the *in vitro* experiments, osteoarthritic reactions were induced in human chondrocytes by incubation with interleukin, which strongly stimulated hyaluronan synthesis. This increased synthesis rate could be reversed by the broad-spectrum inhibitor valspodar selectively, as no reduction of general PG synthesis or deposition of PG in the territorial area of chondrocytes in the alginate beads could be detected. Valspodar also reduced the amount of hyaluronan and similarly of PG on chondrocytes. This observation confirmed our hypothesis that hyaluronan was mainly responsible for anchoring PG to the chondrocyte cell surface[5].

Inhibition of hyaluronan synthesis by valspodar reduced and even normalized the loss of PG from osteoarthritic bovine cartilage slices in organ culture. Analysis of a set of ABC transporter inhibitors revealed that the hyaluronan export inhibitors valspodar and verapamil were superior to other inhibitors. An interesting additional observation is that the hyaluronan export inhibitors, particularly nicardipine, left the PG concentrated around the cells, e.g., at the site where they are synthesized. This result suggested that overproduction of hyaluronan in osteoarthritic cartilage significantly contributed to PG loss. The results again confirmed the importance of a balanced metabolism of hyaluronan and aggrecan for cartilage integrity[30]. Undoubtedly, the synthesis of link protein and collagens and proteolytic degradation also contribute to osteoarthritic cartilage loss, but these are secondary events following stimulation of hyaluronan production synthesis[20,21]. It is therefore unlikely that the hyaluronan export inhibitor valspodar caused protection from aggrecan loss by a direct effect on link protein or proteases.

It is still unclear how increased hyaluronan production reduces the PG level in cartilage. Several mechanisms are conceivable. Excess of hyaluronan could attract water into the cartilage and increase swelling, allowing reduced restriction of the diffusion of proteases that degrade collagens and aggrecan. A less likely possibility is the direct inhibition of proteases, because they are not the prime targets of ABC transporter inhibitors at the applied concentrations. Alternatively, it could lead to displacement of preformed

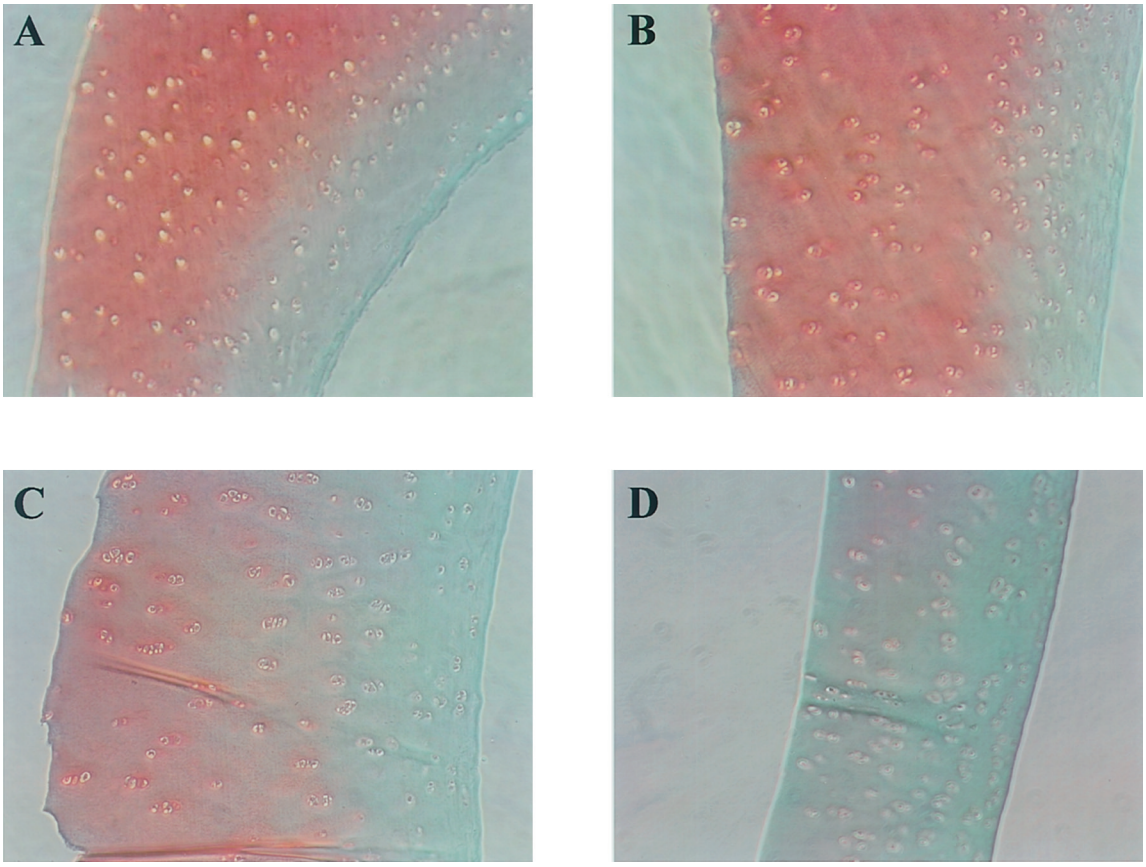

Figure 4. Histologic analyses of bovine articular cartilage cultured with 5 ng/ml IL-1ß and 12 µM of the MDR inhibitors verapamil (A), nimodipine (B), nicardipine (C), or bepridil (D). Proteoglycans were stained in thin sections with safranin-O and counterstained with fast green.

aggregates of hyaluronan and aggrecan in the matrix that as a result diffuse out of the cartilage without proteolytic degradation. Reduced hyaluronan concentration on the chondrocyte matrix might also be a signal for the chondrocyte to reduce the synthesis of proteases. Since some inhibitors were Ca^{2+} channel blockers, they could reduce hyaluronan synthesis through intracellular signalling cascades. But this possibility also appears unlikely, because our previous studies have shown that valspodar and other inhibitors at the applied concentrations selectively blocked hyaluronan synthase activity as well as hyaluronan export in isolated membranes, indicating that the 2 activities were coupled[8]. It is therefore likely that prevention of PG loss was due to direct inhibition of hyaluronan export rather than inhibition of matrix metalloproteases. But as a secondary effect, it cannot be excluded that reduced hyaluronan concentrations prevent the access of protease to their substrates in cartilage.

Inhibition of hyaluronan synthesis and its effects on PG retention has been analyzed using HAS-2 antisense oligonucleotides on chondrocytes and cartilage slices in cell cultures that were not stimulated by IL-1[31]. In contrast to the current study on IL-1 stimulated cartilage, these investigators found that inhibition of hyaluronan synthesis caused enhanced PG release from chondrocytes or cartilage. This confirms the notion that well balanced synthesis rates of hyaluronan and PG are required to maintain cartilage integrity[30].

Verapamil was evaluated as a hyaluronan export inhibitor in a rat model of osteoarthritis. It was able to prevent proteoglycan loss from osteoarthritic rat knees. It is therefore possible that inhibitors of hyaluronan export may reduce or prevent osteoarthritis in humans.

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REFRENCES

1. Kuettner KE, Thonar EJMA. Cartilage integrity and homeostasis. In: Klippel JH, Dieppe PA, editors. Rheumatology. 2nd ed. London: Mosby; 1998:6.1-6.16.
2. Johnsson C, Hallgren R, Elvin A, Gerdin B, Tufveson G. Hyaluronidase ameliorates rejection-induced edema. Transpl Int 1999;12:235-43.
3. Johnsson C, Hallgren R, Tufveson G. Role of hyaluronan in acute pancreatitis. Surgery 2000;127:650-8.

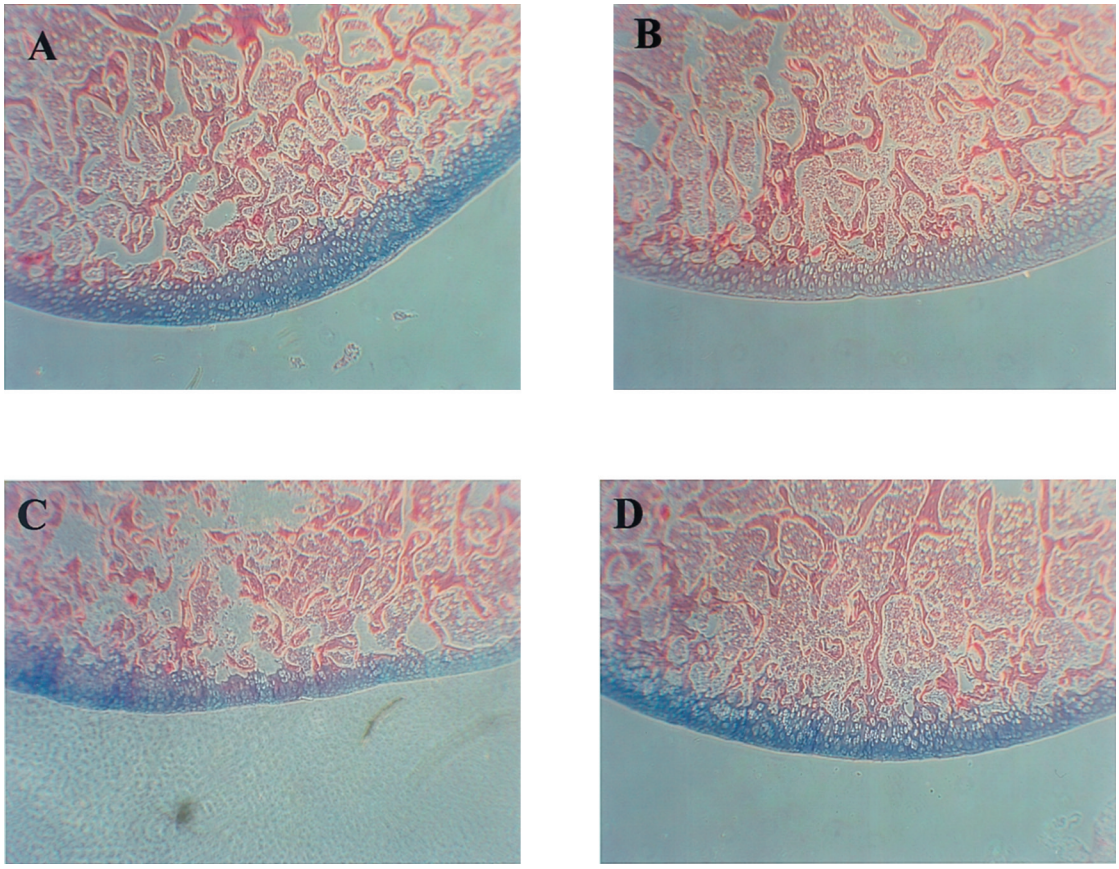

Figure 5. Histological sagittal sections of rat knee joints on Day 17 after intraarticular injection of saline (A, C), or 0.1 mg iodoacetate (B, D). The rats received drinking water only (A, B) or verapamil in drinking water for 17 days (C, D). PG were stained in thin sections of the tibia with alcian blue and counterstained with nuclear red.

4. Goransson V, Johnsson C, Jacobson A, Heldin P, Hallgren R, Hansell P. Renal hyaluronan accumulation and hyaluronan synthase expression after ischaemia-reperfusion injury in the rat. Nephrol Dial Transplant 2004;19:823-30.
5. Prehm P. Mechanism, localization, and inhibition of hyaluronate synthesis. In: Kuettner K, editor. Articular cartilage biochemistry. New York: Raven Press; 1986:81-91.
6. Prehm P. Hyaluronate is synthesized at plasma membranes. Biochem J 1984;220:597-600.
7. Ouskova G, Spellerberg B, Prehm P. Hyaluronan release from Streptococcus pyogenes: Export by an ABC transporter. Glycobiology 2004;14:931-8.
8. Prehm P, Schumacher U. Inhibition of hyaluronan export from human fibroblasts by inhibitors of multidrug resistance transporters. Biochem Pharmacol 2004;68:1401-10.
9. Prehm P. Identification and regulation of the eukaryotic hyaluronate synthase. Ciba Found Symp 1989;143:21-30.
10. Prehm P. Hyaluronan, In: Steinbüchel A, editor. Biopolymers. Weinheim, Germany: Whiley-VCH-Verlag; 2002:379-406.
11. Poole CA, Matsuoka A, Schofield JR. Chondrons from articular cartilage. III. Morphologic changes in the cellular microenvironment of chondrons isolated from osteoarthritic cartilage. Arthritis Rheum 1991;34:22-35.
12. Hamerman D, Sasse J, Klagsbrun M. A cartilage-derived growth factor enhances hyaluronate synthesis and diminishes sulfated glycosaminoglycan synthesis in chondrocytes. J Cell Physiol 1986;127:317-22.
13. Smith RL. Degradative enzymes in osteoarthritis. Front Biosci 1999;4:D704-12.
14. Chevalier X. Upregulation of enzymatic activity by interleukin-1 in osteoarthritis. Biomed Pharmacother 1997;51:58-62.
15. Jiang H, Peterson RS, Wang W, Bartnik E, Knudson CB, Knudson W. Requirement for the CD44 cytoplasmic domain for hyaluronan binding, pericellular matrix assembly, and receptor-mediated endocytosis in COS-7 cells. J Biol Chem 2002;277:10531-8.
16. D'Souza AL, Masuda K, Otten LM, Nishida Y, Knudson W, Thonar EJ. Differential effects of interleukin-1 on hyaluronan and proteoglycan metabolism in two compartments of the matrix formed by articular chondrocytes maintained in alginate. Arch Biochem Biophys 2000;374:59-65.
17. Knudson CB, Nofal GA, Pamintuan L, Aguiar DJ. The chondrocyte pericellular matrix: a model for hyaluronan-mediated cell-matrix interactions. Biochem Soc Trans 1999;27:142-7.
18. Chow G, Nietfeld JJ, Knudson CB, Knudson W. Antisense inhibition of chondrocyte CD44 expression leading to cartilage chondrolysis. Arthritis Rheum 1998;41:1411-9.
19. Nishida Y, D'Souza AL, Thonar EJ, Knudson W. Stimulation of hyaluronan metabolism by interleukin-1α in human articular cartilage. Arthritis Rheum 2000;43:1315-26.
20. Kozaci LD, Buttle DJ, Hollander AP. Degradation of type II collagen, but not proteoglycan, correlates with matrix metalloproteinase activity in cartilage explant cultures. Arthritis Rheum 1997;40:164-74.
21. Billinghurst RC, Wu W, Ionescu M, et al. Comparison of the

degradation of type II collagen and proteoglycan in nasal and articular cartilages induced by interleukin-1 and the selective inhibition of type II collagen cleavage by collagenase. Arthritis Rheum 2000;43:664-72.
22. Greenwald RA. Thirty-six years in the clinic without an MMP inhibitor. What hath collagenase wrought? Ann NY Acad Sci 1999;878:413-9.
23. Elliott S, Cawston T. The clinical potential of matrix metalloproteinase inhibitors in the rheumatic disorders. Drugs Aging 2001;18:87-99.
24. Robbins JR, Thomas B, Tan L, et al. Immortalized human adult articular chondrocytes maintain cartilage-specific phenotype and responses to interleukin-1ß. Arthritis Rheum 2000;43:2189-201.
25. Stern M, Stern R. An ELISA-like assay for hyaluronidase and hyaluronidase inhibitors. Matrix 1992;12:397-403.
26. Chandrasekhar S, Esterman MA, Hoffman HA. Microdetermination of proteoglycans and glycosaminoglycans in the presence of guanidine hydrochloride. Anal Biochem 1987;161:103-8.
27. Terry DE, Chopra RK, Ovenden J, Anastassiades TP. Differential use of Alcian blue and toluidine blue dyes for the quantification and isolation of anionic glycoconjugates from cell cultures: application to proteoglycans and a high-molecular-weight glycoprotein synthesized by articular chondrocytes. Anal Biochem 2000;285:211-9.
28. Samnegard E, Cullen DM, Akhter MP, Kimmel DB. No effect of verapamil on the local bone response to in vivo mechanical loading. J Orthop Res 2001;19:328-36.
29. Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P. Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility, morphology, and biochemistry. Arthritis Rheum 1997;40:1670-9.
30. Morales TI, Hascall VC. Correlated metabolism of proteoglycans and hyaluronic acid in bovine cartilage organ cultures. J Biol Chem 1988;263:3632-8.
31. Nishida Y, Knudson CB, Nietfeld JJ, Margulis A, Knudson W. Antisense inhibition of hyaluronan synthase-2 in human articular chondrocytes inhibits proteoglycan retention and matrix assembly. J Biol Chem 1999;274:21893-9.