Effect of Glycosaminoglycans on Matrix Metalloproteinases in Type II Collagen–Induced Experimental Arthritis

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Matrix metalloproteinases (MMPs) are a family of neutral proteinases that are involved in tissue remodeling by mediating degradation of extracellular matrix components in both physiology and pathology. As MMPs appear to play a key role in the degradation of cartilage matrix in the progression of arthritic disease, MMPs are considered as potential therapeutic targets. The effect of chondroitin sulfate A (CSA) on MMPs in type II collagen–induced experimental arthritis was studied. The antiarthritic effect of CSA was evidenced by a decrease in marker activities like lysosomal β-hexosaminidase and β-glucuronidase. Arthritic animals showed significantly higher activity of MMP2 and MMP9 and increased levels of other MMPs, including MMP3 and MT-1 MMP in cartilage and serum. Treatment with CSA significantly decreased the activity of MMPs, particularly MMP9 in serum and synovial effusate and cartilage. The effect of CSA was further studied by fragmenting CSA into low-molecular-weight oligosaccharides. The oligosaccharide-treated animals showed considerably lower MMP activity (particularly MMP9) compared with arthritic controls. The CSA (and the oligosaccharides derived from it) not only reduced the activity of MMPs but also decreased the protein level expression of MMPs, indicating that the production of MMPs is affected. These results indicate that the antiarthritic effect of CSA involves down-regulation of MMPs, which are critically involved in the progression of arthritic disease. Exp Biol Med 232:629–637, 2007

Key words: MMP2; MMP3; MMP9; MT-1 MMP; synovial effusate; CSA; oligosaccharides; experimental arthritis

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Introduction

Arthritis is the most common form of chronic inflammatory disease that leads to impaired joint function. Degradation and remodeling of the extracellular matrix in cartilage are key events in the development of arthritis. Increase in the activity of proteinases, particularly matrix metalloproteinases (MMPs), has been suggested to be one of the factors contributing to cartilage destruction in arthritis (1). Proinflammatory cytokines and growth factors released within the joint act on cells present to produce these enzymes (2). Increase in the activity of MMPs has been found in synovial fluid of osteoarthritic (OA) patients (3). Studies on antibody-induced experimental arthritis showed an increase in the activity of MMP2 and MMP9 (4). The consequence of increase in the proteolytic activity of MMPs was revealed by the presence of soluble cell adhesion molecules and fragments of matrix components like fibronectin (FN) in plasma and synovial fluid (5–8).

A number of nonsteroidal anti-inflammatory drugs and glucocorticoids are currently being employed in the medical management of arthritis (9–11). Although heteropolysaccharides like hyaluronic acid and chondroitin sulfate A (CSA) have been suggested to be useful in the management of arthritis, the mechanism of their action is not clearly understood. These exogenously administered heteropolysaccharides (or monosaccharides like glucosamine sulfate) were considered to provide a precursor for synthesis of articular cartilage (12). It has been suggested that CSA may exert its therapeutic effect by reducing the level of free radicals (13). But it is not known whether these heteropolysaccharides or their degradation products affect the arthritic process by influencing the activity of matrix-degrading proteinases.

As MMPs appear to play a key role in the degradation of cartilage matrix in the progression of arthritic disease, MMPs are considered as potential therapeutic targets. Therefore, several synthetic compounds have been tested as inhibitors of MMPs; very few naturally occurring substances have been identified. Earlier reports from our laboratory using isolated enzymes indicated that heteropolysaccharides inhibit MMPs (14). But it is not known

whether any such action occurs *in vivo*. Therefore, the effect of CSA on MMPs in type II collagen–induced experimental arthritis was studied, and the results presented here showed CSA decreased the activity of MMPs.

Materials and Methods

Acrylamide, bisacrylamide, gelatin, Tween 20, Freund's complete adjuvant, and type II collagen, CSA, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, *p*-nitrophenyl-*N*-acetyl-β-D-glucuronide, and assorted polyclonal antibodies were supplied by Sigma Chemical Co., St. Louis, MO. These antibodies included: anti-MMP2 (affinity-isolated polyclonal antibody raised in a rabbit against the N-terminal segment), anti-MMP3 (affinity-isolated polyclonal antibody raised in a rabbit against the N-terminal segment), anti-MMP9 (affinity-isolated polyclonal antibody raised in a rabbit against the C-terminal segment), MT-1 MMP (affinity-isolated polyclonal antibody raised in a rabbit against the hinge region), TIMP1 (affinity-isolated polyclonal antibody raised in a rabbit against the C-terminal segment), and TIMP2 (affinity-isolated polyclonal antibody raised in a rabbit against the N-terminal segment).

Fragmentation of CSA. CSA (20 mg/ml) was digested with sheep testicular hyaluronidase (1 mg/ml). An aliquot of the digested sample was loaded on Sephadex G-25 column and eluted with phosphate buffer pH 6.0 (15). The smallest active fractions were collected, concentrated, and an aliquot was loaded on Bio-gel-P-10 column and eluted with phosphate buffer pH 6.0. The low molecular weight fraction appeared to have a molecular size of about 5000 Daltons, which corresponds to an oligosaccharide containing 11 repeating disaccharide units.

Induction of Arthritis. Arthritis was induced in male Wistar rats, average body weight of 175–180 g, by injecting an emulsion containing type II collagen and Freund's complete adjuvant at the base of the tail (16). After 21 days, a booster dose was given. One week later, the animals were divided into three groups. Group 1 contained normal controls receiving sterile saline, Group 2 contained arthritic controls, and Group 3 contained arthritic controls that received treatment with CSA. All the groups contained six animals each. Clinical severity of arthritis was assessed by quantification of paw volume changes using a dial gauge caliper. After the induction of arthritis, Group 3 was given CSA at a concentration of 300 μg uronic acid equivalent/100 g body weight po for 2 weeks. To study the effect of oligosaccharides derived from CSA, arthritis was induced in rats by injecting an emulsion containing type II collagen as above. Animals were divided into three groups of six each. Group 1 was normal control receiving sterile saline. Group 2 was arthritic control and Group 3 was arthritic animals treated with oligosaccharide. Twenty-one days after injection, a booster dose was given to animals. After one week, Group 3 animals were fed with 300 μg uronic acid equivalent oligosaccharide/100 g body weight for 2 weeks. At the end of the experiment, the animals were sacrificed under anesthesia and synovial effusate, blood, and cartilage were collected to study the activity of various enzymes. Cartilage was homogenized at 4°C (1 g/100 ml homogenate in 0.05 *M* phosphate buffer, 0.1 *M* NaCl, 0.05% Tween 20, pH 7.2) and centrifuged, and the supernatant was used for the analysis.

Extraction of MT-1 MMP. Cartilage was homogenized with 0.5% sodium dodecyl sulfate, kept overnight at 4°C, and centrifuged. The final volume was made up to 0.05% with phosphate-buffered saline.

Assay of Lysosomal Enzymes. Lysosomal enzymes, such as β-glucuronidase (17) and β-hexosaminidase in serum and cartilage, were assayed as described earlier (18).

Zymography. MMPs were assayed by zymography using 7.5% polyacrylamide copolymerized with gelatin (2 mg/ml) as described earlier (19). After electrophoresis, the gels were washed and incubated in a substrate buffer containing 50 m*M* Tris-HCl/5 m*M* $CaCl_2$ pH 7.5. The gels were stained with Coomassie blue stain and then de-stained. Intensity of the bands was measured using a Bio-Rad Gel Doc using QuantityOne 4.5.0.

ELISA. The level of the enzyme protein was determined by ELISA (20). After treatment with horseradish peroxidase–conjugated secondary antibody (1:1000) for 1 hr at room temperature, *o*-dianisidine reagent was added and absorbance was measured at 405 nm.

Succinylated Gelatin Assay. MMP2 and MMP9 were assayed colorimetrically using succinylated gelatin as substrate (21). MMP2 was immunoprecipitated by the addition of antibody against MMP2, and the supernatant was utilized for the assay of MMP9 by using 50 m*M* borate buffer pH 8.5 containing 10 m*M* $CaCl_2$. MMP9 was immunoprecipitated in the supernatant by using specific antibody and the activity of MMP2 in the supernatant was determined using borate buffer pH 7.0 containing 10 m*M* $CaCl_2$. Reaction was stopped by the addition of 0.03% trinitrobenzene sulfonic acid. The O.D. was measured at 450 nm.

Statistical Analysis. Statistical analyses were done using one-way ANOVA followed by Duncan's post-hoc test to identify the differences and Levene's *t* test using SPSS for Windows, version 10.0 (SPSS Inc., Chicago). Differences of $P < 0.05$ were considered to be significant.

Results

Effect of CSA on Experimental Arthritis. To study the effect of CSA *in vivo*, arthritis was induced in rats. The induction of arthritis was evidenced by edema of the paws (Table 1) and changes in the activities of marker enzymes. There was a significant decrease in edematous paw volume in arthritic animals receiving CSA (Table 2). Biochemical markers such as β-D-glucuronidase, β-hexosaminidase, and super oxide dismutase (SOD) in serum and

Table 1. Induction of Arthritis: Changes in Paw Size (mm)[a]

	Days, *n*							
Group	0	4	8	12	16	20	24	28
Control	5.13 ± 0.03	5.13 ± 0.03	5.2 ± 0.05	5.2 ± 0.05	5.2 ± 0.05	5.3 ± 0.03	5.3 ± 0.03	5.3 ± 0.03
Arthritic	5.13 ± 0.03	5.13 ± 0.03	5.5 ± 0.05	5.6 ± 0.06	5.6 ± 0.43	5.6 ± 0.23	6.4 ± 0.23*	6.5 ± 0.01*

[a] Paw sizes of normal controls and arthritic animals were measured at regular intervals. Values given are the mean of 5 experiments ± SEM.
* Statistically significant compared with controls ($P < 0.01$).

cartilage were assayed; the results are shown in Figures 1 and 2. An increase in the activity of these enzymes was observed in both serum and cartilage of arthritic animals compared with normal controls. In animals treated with CSA, there was a significant decrease in the activity of the lysosomal enzymes in both cartilage and serum when compared with arthritic animals, indicating antiarthritic effect of CSA. CSA treatment did not, however, show any effect on the activity of SOD.

Changes in the Activity of MMPs in Experimental Arthritic Animals Treated with CSA. The activity of MMPs in synovial effusate, serum, and cartilage was assayed by zymography (Fig. 3A). The gelatin-degrading activity increased significantly in the arthritic group compared with the normal controls, but there was a significant decrease in gelatinolytic activity in the CSA-treated group. Gelatinolytic activity was further quantified by measuring the intensity of the bands (Fig. 3B).

The activity of MMP was also measured by using succinylated gelatin as substrate (Fig. 4). The activity of MMP9 in synovial effusate was significantly decreased in the CSA-treated group compared with arthritic controls. Both MMP2 and MMP9 decreased in the serum of arthritic animals treated with CSA. In cartilage, there was no significant change in the activity of MMP2, but MMP9 was significantly lowered in CSA-treated animals when compared with the untreated arthritic control.

The protein-level expression of different enzymes (such as MMP2, MMP3, and MMP9) in serum was also determined by ELISA (Fig. 5). The amount of all these MMPs decreased significantly in the CSA-treated group compared with arthritic controls. Similarly, the levels of MMP2, MMP3, MMP9, and MT-1 MMP, as determined by ELISA in cartilage, showed significant decreases in CSA-treated animals compared with arthritic controls.

Effect of CSA on the Level of TIMPs in Experimental Arthritis. To study whether treatment with CSA has any effect on the endogenous inhibitors of MMPs, the levels of these inhibitors in serum and cartilage of the experimental animals were assayed by ELISA. The results are shown in Figure 6. Serum levels of TIMP1 and TIMP 2 and cartilage levels of TIMP2 were significantly decreased in arthritic animals treated with CSA. The level of TIMP1 in serum was very low.

Effect of Oligosaccharides Derived from CSA on Experimental Arthritis. The effect of CSA was investigated further by fragmenting it into low-molecular-weight oligosaccharides by degradation with testicular hyaluronidase. The oligosaccharides were fractionated and the low molecular weight fractions corresponding to about 5000 Dalton size was isolated, concentrated, and used to study its effect on MMPs in arthritis. Oligosaccharide was administered to experimental animals in which arthritis was induced. Upon treatment with oligosaccharides derived from CSA, significant reduction in paw volume was observed (Table 3).

Arthritic animals treated with oligosaccharide showed a significant decrease in the activity of both β-glucuronidase and β-hexosaminidase in serum. But SOD, which increased in arthritis, was not affected by the administration of oligosaccharides derived from CSA (Fig. 7).

Changes in the Activity of MMPs in Experimental Arthritic Animals Treated with Oligosaccharides Derived from CSA. The activity of MMPs in synovial effusate, serum, and cartilage of experimentally arthritic animals receiving oligosaccharides derived from CSA was studied by succinylated gelatin (Fig. 8). MMP9 activity was

Table 2. Protective Effect of CSA: Changes in Paw Size (mm)[a]

	Days, *n*				
Group	0	4	8	12	16
Control	5.30 ± 0.03	5.30 ± 0.03	5.33 ± 0.03	5.34 ± 0.03	5.34 ± 0.03
Arthritic	6.5 ± 0.01	6.5 ± 0.006	6.51 ± 0.003	6.52 ± 0.005	6.51 ± 0.006
Arthritic + CSA	6.53 ± 0.03	6.4 ± 0.10	6.15 ± 0.02*	5.88 ± 0.01*	5.41 ± 0.11*

[a] Paw sizes of normal controls, arthritic animals, and arthritic animals treated with chondroitin sulfate were measured at regular intervals. Values given are mean of 5 experiments ± SEM.
* Statistically significant compared with arthritic controls ($P < 0.01$).

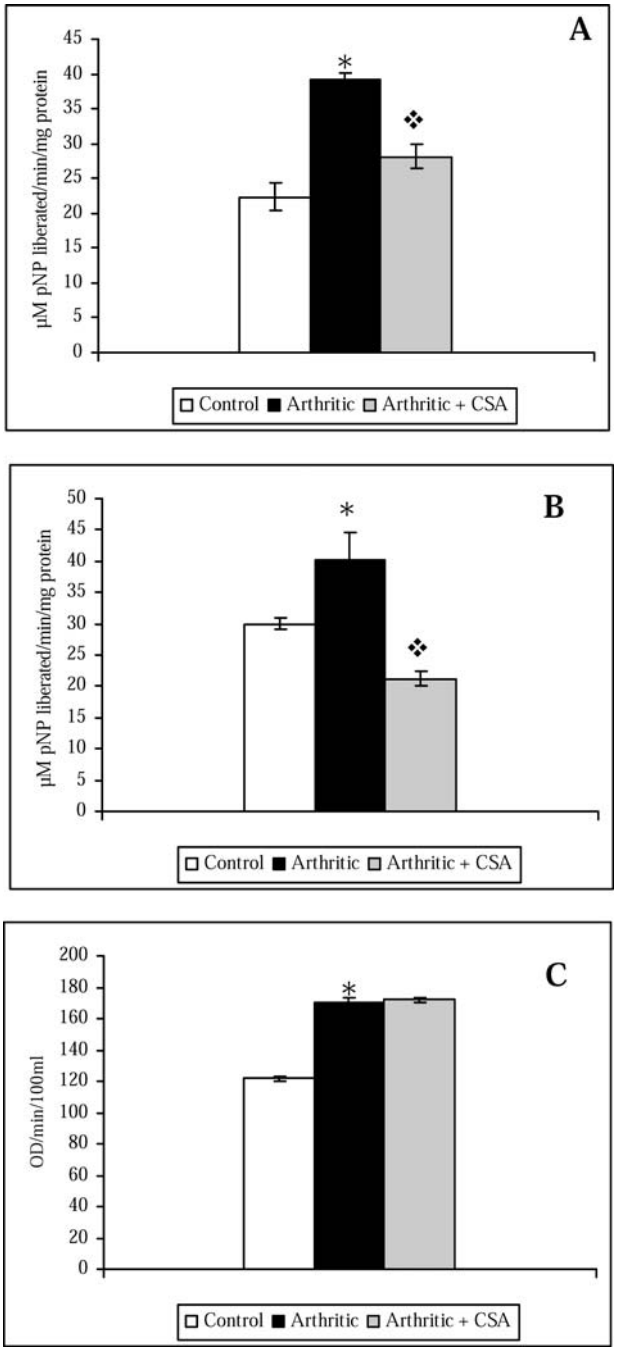


Figure 1. Changes in activities of lysosomal enzymes and SOD in serum of experimental arthritic animals. (A) Sera of experimental animals were used for the assay of β-glucuronidase using *p*-nitrophenyl-*N*-acetyl-β-D-glucuronide as substrate. (B) β-hexosaminidase was assayed using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate. (C) SOD activity was also assayed. Values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic controls ($P < 0.01$).

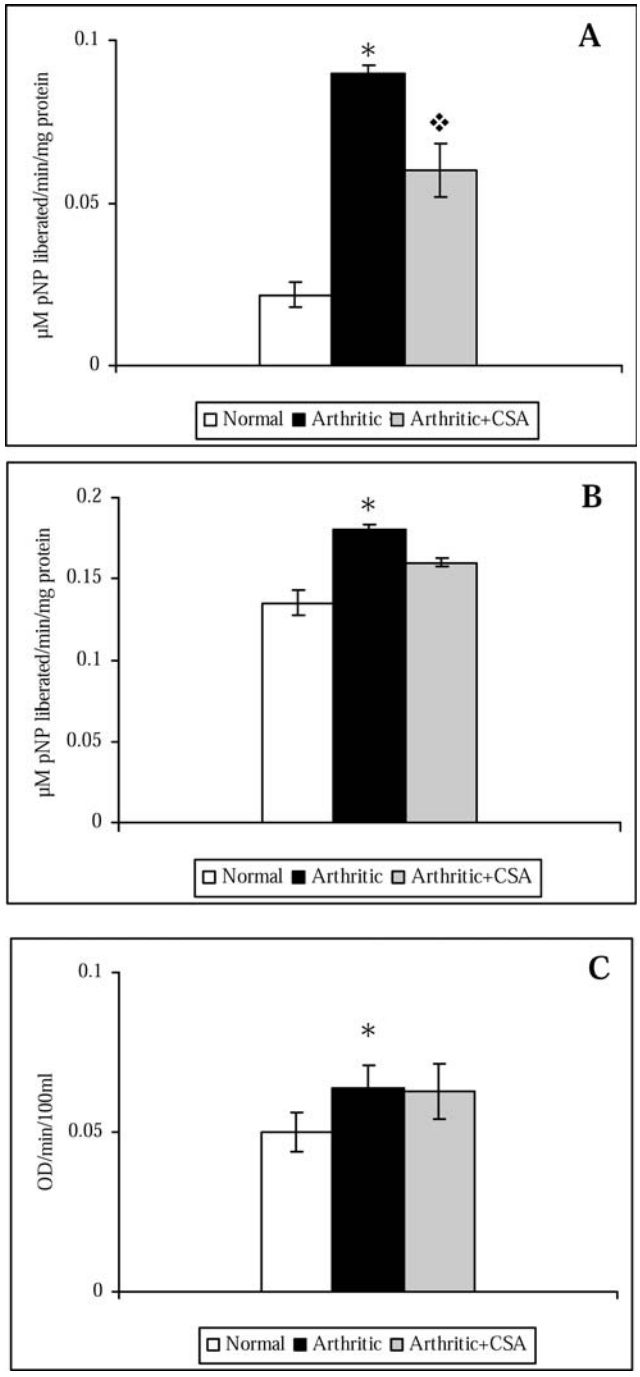


Figure 2. Changes in the activities of lysosomal enzymes and SOD in cartilage of experimental animals. Cartilage extracts of experimental animals were used for the assay of β-glucuronidase (A), β-hexosaminidase (B), and SOD (C) activity as described under Methods. Values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic controls ($P < 0.01$).

found to be decreased in the serum, synovial effusate, and cartilage of oligosaccharide-treated animals compared with arthritic controls. Although activity of MMP2 was considerably decreased in the serum and cartilage of the oligosaccharide-treated group, no decrease in synovial effusate activity was seen in this group. The protein-level expression of MMPs in experimental animals was also studied by ELISA (Fig. 9). In serum and cartilage, the level of MMP2, MMP3, and MMP9 were significantly decreased in the oligosaccharide-treated group compared with arthritic

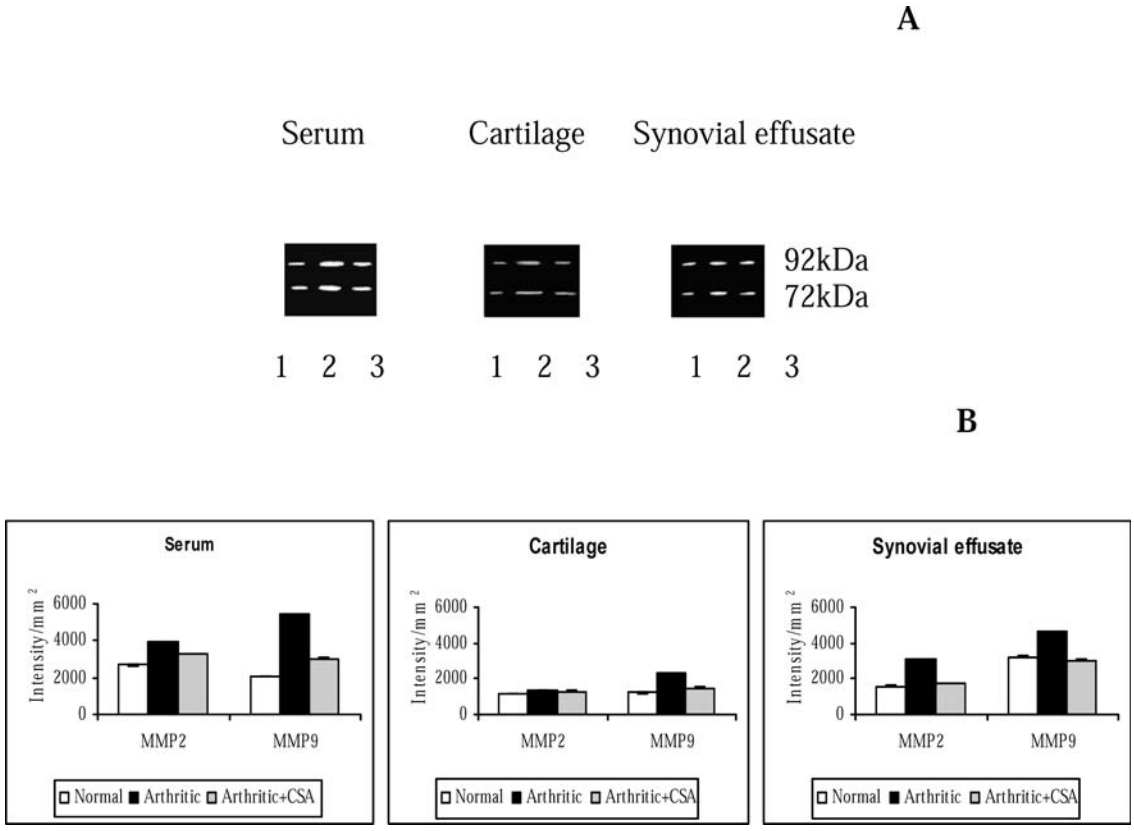


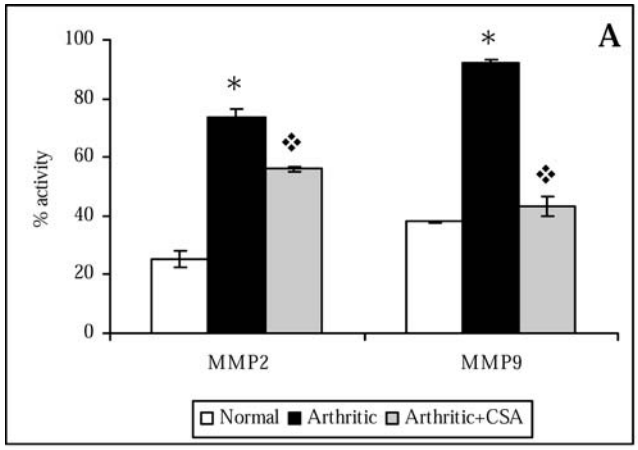
Figure 3. Activity of MMPs in experimental animals with arthritis. Serum, cartilage, and synovial effusates of experimental animals were subjected to zymography. Zymogram gels consist of 7.5% acrylamide copolymerized with 2 mg/ml gelatin. Gels were stained with Coomassie blue stain for 1 hr and de-stained. Saline-treated animals served as controls. The activity of MMP appeared as white bands (A), which correspond to 92 kDa and 72 kDa. Lane 1, normal controls; Lane 2, arthritic controls; Lane 3, arthritic animals receiving CSA. After zymography, the intensity of each band was analyzed in the Bio-Rad Gel Documentation System using QuantityOne 4.5.0 software (B). The values are expressed as intensity/mm^2 $\pm$ SEM.

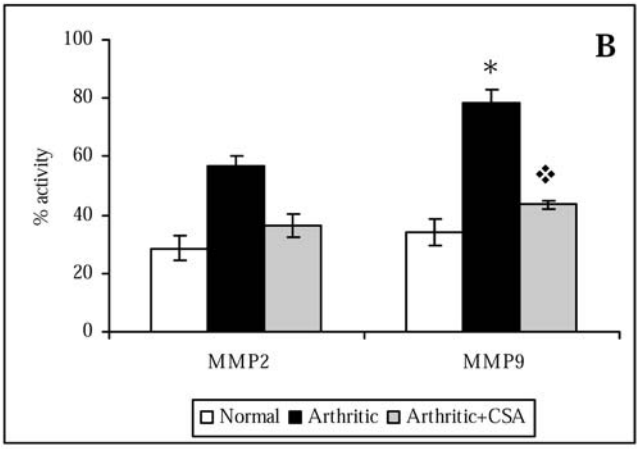
controls. MT-1 MMP expression was also decreased in the oligosaccharide-treated group compared with arthritic control.

Discussion

Several compounds, mostly those classified as non-steroidal anti-inflammatory drugs (NSAID), are being used in the treatment of arthritis. These anti-inflammatory agents cause inhibition of cyclooxygenase, leading to inhibition of production of prostaglandins. Naturally occurring compounds (such as glucosamine) and heteropolysaccharides (such as hyaluronic acid and CSA) are also reported to be of use in the treatment of arthritis (22, 23). The mechanisms by which naturally occurring substances like heteropolysaccharides influence arthritis are not known. Our results indicate that CSA could affect the progression of arthritic disease by its effect on MMPs, which increased in arthritic condition. Oral administration of CSA reduced the severity of arthritic process in type II collagen–induced experimental arthritis. This was evident from the observation of reduced paw volume in experimental arthritic animals treated with CSA and changes in the activity of enzymes, such as lysosomal degradative enzymes, and antioxidant enzymes, such as SOD. CSA administration resulted in a decrease in the activity level of β-glucuronidase and β-hexosaminidase to near-normal levels. These results are in agreement with the earlier observations where histological analysis showed minimal evidence of joint destruction and inflammation with very little synovial hyperplasia in animals treated with chondroitin-4 sulphate (24). Heteropolysaccharides and monosaccharides (such as glucosamine and glucosamine-6-sulfate) have been reported to reduce joint inflammation in OA and rheumatoid arthritis patients (25, 26). Although it is not clear how these heteropolysaccharides act, it appears that they exert anti-inflammatory and antioxidant effects (24). Inflammatory cytokines and reactive oxygen species are reported to accumulate and influence the progression of arthritic disease (13). It has been suggested that CSA may exert its action by influencing the production of inflammatory agents and free radicals (26).

Our results indicate that CSA might exert its effect by inhibiting MMPs, which are critically involved in the matrix degradation occurring during the progression of the disease. The complications of arthritis, where articular cartilage





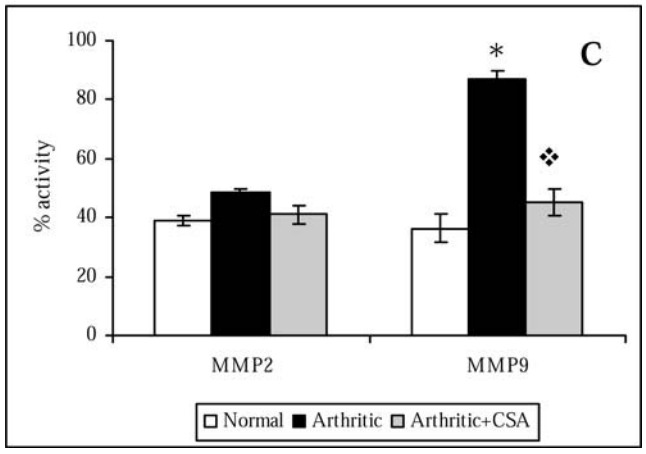
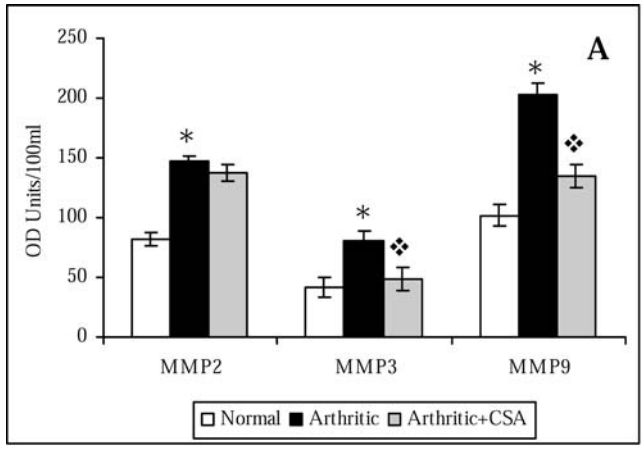


Figure 4. Changes in the activity of MMPs in experimental animals with arthritis. Serum (A), cartilage (B), and synovial effusate (C) were assayed for MMP2 and MMP9 using succinylated gelatin as substrate. After incubating the mixture at 37°C for 3 hrs, 0.03% trinitrobenzenesulfonic acid was added; O.D. was measured at 450 nm. The maximum total gelatinase activity corresponding to MMP9: serum, 0.96 units/100 ml; cartilage, 1.1 units/mg protein; and synovial effusate, 3.57 units/mg protein. The maximum total gelatinase activity corresponding to MMP2: serum, 0.97 units/100 ml; cartilage, 0.08 units/mg protein; and synovial effusate, 0.5 units/mg protein. Values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic animals ($P < 0.01$).



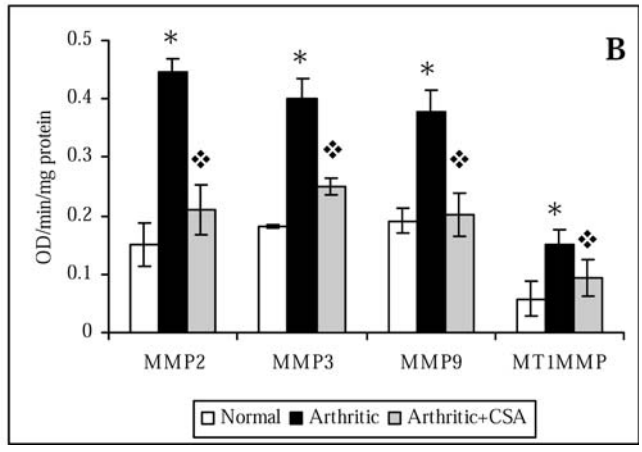


Figure 5. Changes in the level of different MMPs in cartilage and serum of arthritic animals (ELISA). Serum (A) and cartilage extract (B) of experimental animals were coated on a multiwell plate as antigens and subjected to ELISA using specific antibodies against MMP2, MMP3, and MMP9 with *o*-dianisidine as substrate. O.D. was measured at 405 nm. Cartilage was also tested for MT-1 MMP. Values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic controls ($P < 0.01$).

degradation and remodeling occur, are related to proteolytic degradation; the MMPs play a key role in the cartilage degradation. Results of investigations using oligosaccharide derived from CSA also showed an antiarthritic effect. The oligosaccharide-treated animals showed considerably lower MMPs, particularly MMP9 activity levels, compared with arthritic controls. CSA and the oligosaccharide not only decreased the activity of MMPs *in vivo*, but the protein-level expression of MMPs was also found to be lowered, indicating that the production of MMPs is affected. The different MMPs that were affected in the cartilage include MMP2, MMP9, and MT-1 MMP.

Results of the previous investigations on the MMPs in experimental arthritis suggest that MMP3 is probably the key enzyme during the early stages of the disease, causing degradation of the nonfibrillar collagen and other compo-

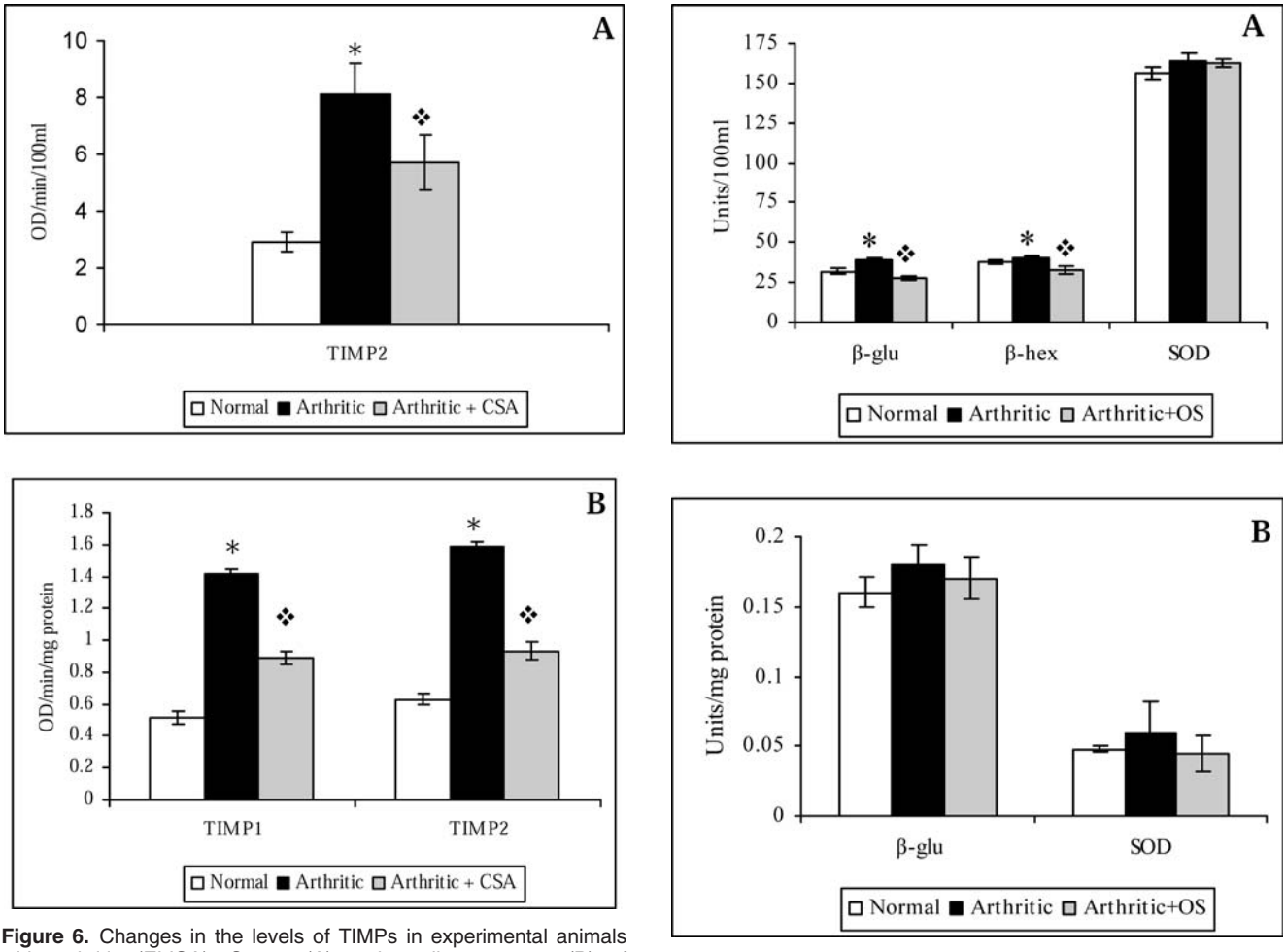


Figure 6. Changes in the levels of TIMPs in experimental animals with arthritis (ELISA). Serum (A) and cartilage extract (B) of experimental animals were coated on a multiwell ELISA plate as antigens and subjected to ELISA using specific antibodies against TIMP1 and TIMP2 with *o*-dianisidine as substrate. O.D. was measured at 405 nm. Values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic controls ($P < 0.01$).

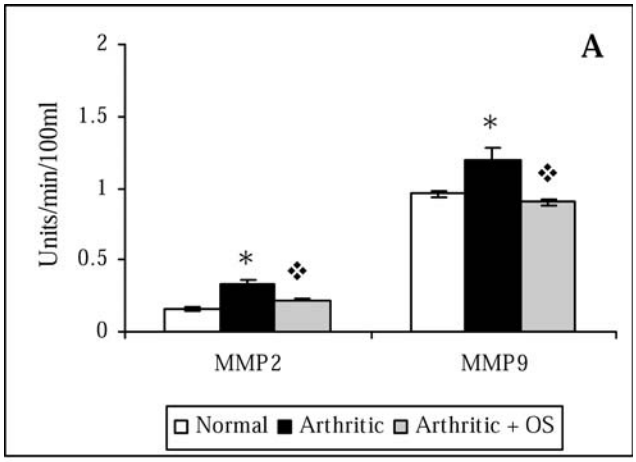
Figure 7. Changes in the activities of lysosomal enzymes and SOD in serum and cartilage of experimental animals treated with oligosaccharides. Serum (A) and cartilage extract (B) of normal controls, arthritic animals, and arthritic animals receiving oligosaccharides were used for the assay of β-glucuronidase, using *p*-nitrophenyl-*N*-acetyl-β-D-glucuronide as substrate and β-hexosaminidase using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate (one unit of activity expressed as μ*M* pNP liberated/min). SOD (one unit of activity expressed as a shift in O.D./min) was assayed as described under Methods. The values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic controls ($P < 0.01$).

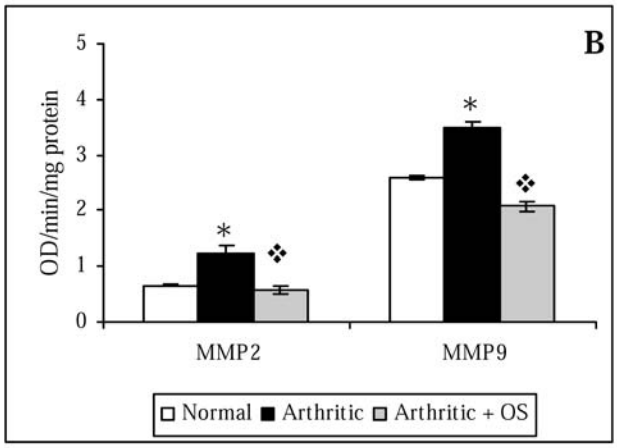
nents, such as the proteoglycans of the extracellular matrix in cartilage. Gelatinases such as MMP2 and MMP9, which act on collagen types IV and V (denatured collagen fragments), appear at later stages in the progression of the disease. MT-1 MMP, which is a key cell surface membrane–type MMP involved in altering cell matrix interaction and activating other MMPs, increases with

Table 3. Protective Effect of Oligosaccharides: Changes in Paw Size with Treatment (mm)[a]

	Days, *n*				
Group	0	4	8	12	16
Control	5.20 ± 0.03	5.23 ± 0.03	5.23 ± 0.03	5.24 ± 0.03	5.26 ± 0.03
Arthritic	6.60 ± 0.01	6.60 ± 0.006	6.61 ± 0.003	6.62 ± 0.005	6.6 ± 0.006
Arthritic + oligosaccharides	6.53 ± 0.14	6.13 ± 0.02	6.07 ± 0.03*	5.56 ± 0.01*	5.33 ± 0.04*

[a] Paw sizes of normal controls, arthritic animals, and arthritic animals treated with oligosaccharides were measured at regular intervals. Values given are mean of 5 experiments ± SEM.
* Statistically significant compared with arthritic controls ($P < 0.01$).





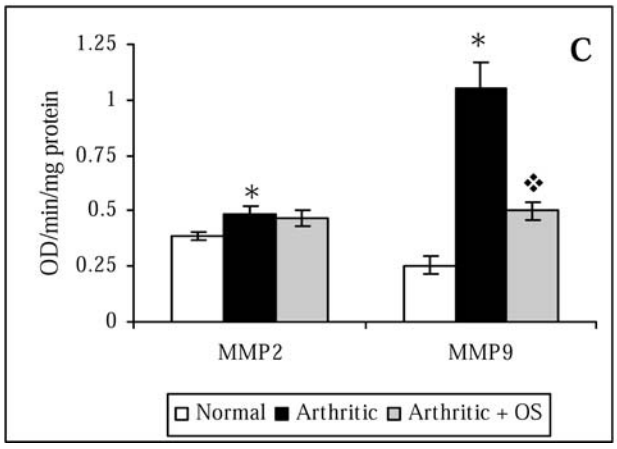
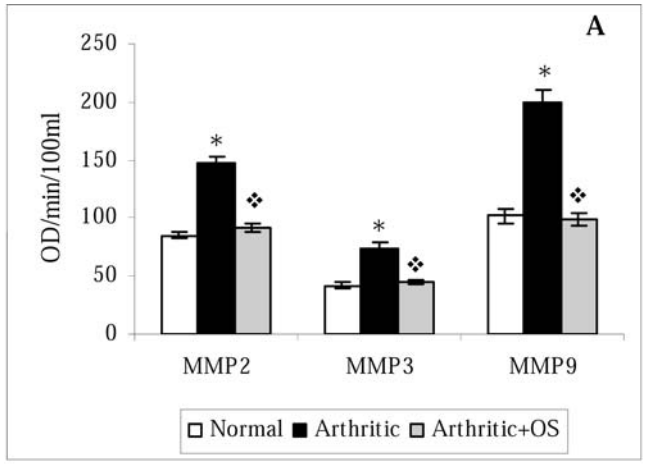


Figure 8. Changes in the activity of MMPs in experimental animals treated with oligosaccharides. Serum (A), cartilage (B), and synovial effusate (C) of normal controls, arthritic animals, and arthritic animals treated with oligosaccharides were assayed for MMP2 and MMP9 using succinylated gelatin as substrate. The values given are the average of 5 experiments ± SEM. * Statistically significant compared with controls ($P < 0.01$). ❖ Statistically significant compared with arthritic controls ($P < 0.01$).



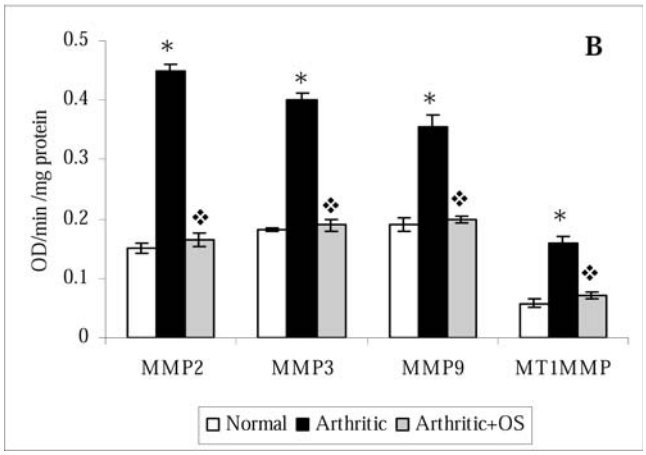


Figure 9. Changes in the levels of MMPs in experimental animals treated with oligosaccharides (ELISA). Serum (A) and cartilage extract (B) of normal controls (Group 1), arthritic animals (Group 2), and arthritic animals treated with oligosaccharides (Group 3) were coated on a multiwell ELISA plate as antigens and subjected to ELISA using specific antibodies against MMP2, MMP3, MMP9, and MT-1 MMP and developed using *o*-dianisidine as substrate. O.D. was measured at 405 nm. The values given are the average of 5 experiments ± SEM. * Statistically significant compared with Group 1 ($P < 0.01$). ❖ Statistically significant compared with arthritic group 2 ($P < 0.01$).

MMP3 during the early stages. Substrates for MT-1 MMP include receptors and matrix proteins like proteoglycans and other components of the matrix. Increases in the level of MT-1 MMP during early stages suggest that it may also trigger alteration in cell–matrix interaction during the early stages.

Our results show that use of an MMP inhibitor decreases the severity of experimental arthritis. Treatment with CSA caused a decrease in the activity of MMPs. Of the different MMPs, the maximum effect was produced on MMP9. Decreases in MMP activity and decreases in MMP level (ELISA) after treatment with CSA suggest reduced production of MMPs by chondrocytes, synovial cells, and blood cells. Because MMP activity seems to increase with inflammatory conditions (27), overproduction of inflamma-

tory cytokines may be the cause. Certain anti-inflammatory agents reduce the expression of MMPs (28). It is also reported that CSA may act as an anti-inflammatory agent (29). CSA may inhibit the overproduction of inflammatory cytokines, which in turn may reduce the expression of MMPs. Fragmentation of polysaccharides to oligosaccharides increases their absorption and bioavailability. Decrease in the production of MMPs in oligosaccharide-treated arthritic animals also suggests that the degradation products of CSA retain their ability to reduce the activity of MMPs and therefore the degradation of cartilage. These results on the inhibition of MMPs and the reduction in the severity of arthritis suggest that CSA-derived oligosaccharides have the potential to serve as antiarthritics.

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