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Original Article

Effects of chondroitin sulfate on modulation of MMPs and TIMP-1 in a monosodium iodoacetate-induced osteoarthritis model

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Abstract: Osteoarthritis (OA) is caused by damage to cartilage and chondrocytes and is characterized by inflammatory cytokine secretion during disease progression. Secreted cytokines regulate the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), which are involved in chondrocyte repair. The glycosaminoglycan chondroitin sulfate (CS), a component of various connective tissues, stimulates chondrocytes to regenerate cartilage and prevents destruction. CS is currently being used in clinical practice to treat OA, but the mechanism by which CS influences OA is unclear. In this study, the effect of CS on MMPs and TIMP-1 was investigated in OA animal models. The OA models were treated with 300, 600, and 900 mg/kg CS for 6 weeks. After 2 weeks of treatment, OA was induced by the injection of monosodium iodoacetate into the knee joint capsule of Sprague-Dawley rats. Serum levels of MMPs and TIMP-1 were measured by an enzyme-linked immunosorbent assay and mRNA levels were quantitated by quantitative real-time polymerase chain reaction using cartilage tissue isolated from the joints. CS (900 mg/kg) significantly down-regulated the levels of MMP-2 (-27%), MMP-3 (-19%), MMP-9 (-40%), and MMP-13 (-15%) and the mRNA levels of *MMP-2* (-29%), *MMP-3* (-15%), *MMP-9* (-29%), and *MMP-13* (-17%). CS also significantly up-regulated TIMP-1 at the mRNA (28%) and protein (24%) level. Histopathologic and radiographic analyses showed that the cartilage and synovium were effectively preserved. Accordingly, CS regulates *MMP* and *TIMP-1* levels, thereby reducing decomposition of the chondrocyte matrix and protecting chondrocytes. Thus, CS can be used to treat OA.

Keywords: Osteoarthritis, chondroitin sulfate, monosodium iodoacetate, matrix metalloproteinase, and tissue inhibitor of metalloproteinase

Introduction

Osteoarthritis (OA) is the most common cause of joint pain, affecting about 15% of the population, and is considered a major public health problem worldwide. It is an inflammatory disease that is common in the elderly and women, causing joint and cartilage damage [1-3].

Recent studies have shown that OA progression is associated with the secretion of inflammatory cytokines, which increase matrix metalloproteinase (MMP) activity [4, 5]. MMPs cause severe damage to cartilage and chondrocytes via degradation of collagen and proteoglycans, components of cartilage [6, 7]. Tissue inhibitors of metalloproteinases (TIMPs) block chondrocyte damage by inhibiting MMP activity [8, 9].

Chondroitin sulfate (CS), a glycosaminoglycan, is a major component of the extracellular matrix of many connective tissues, including cartilage, bone, skin, ligaments, and tendons. It enhances homogenization/catabolism balance of the extracellular cartilage matrix, reduces the action factor, and reduces the absorption characteristics of subchondral osteoblasts [10-12]. The anti-inflammatory effects of CS and its ability to reduce MMP-3 and MMP-13 levels in OA induced by interleukin-1β have been demonstrated in several studies [13-19]. However, stud-

ies of cartilage damage and recovery in monosodium iodoacetate (MIA)-induced OA are lacking.

The MIA experimental model involves the destruction of articular cartilage by inducing the persistent infiltration of synoviocytes and inflammatory cells and is commonly used to assess pain associated with OA [20, 21]. This model is widely used for the pharmacological evaluation of new drug treatments [22].

In this study, we hypothesized that CS could down-regulate MMPs and up-regulate TIMP-1 to protect against and repair cartilage damage. Accordingly, we investigated the effect of CS on serum protein levels, mRNA expression levels, and histological properties in the MIA-induced OA model.

Materials and methods

Animals

Male Sprague-Dawley rats (170-200 g, 6 weeks old) were obtained from RaonBio Inc. (Yong-in, Korea). The rats were acclimated for 10 days and housed under controlled conditions with a temperature of 22 ± 2°C, humidity of 55 ± 15%, a 12-hour light/12-hour dark cycle, and fresh-air ventilation (10-15 times/hour). The rats were housed in solid-bottom cages with free access to food and water. All experimental procedures complied with the NIH Guide for the Care and Use of Laboratory Animals and the Korean National Animal Welfare Law. The experimental animal facility and study protocols were approved by the Institutional Animal Care and Use Committee of Daejeon University (DJUARB2017-020).

Test material doses and groups

CS manufactured by Internacional Farmaceutica Argentina S.A. (Buenos Aires, Argentina) was obtained from Ju Yeong NS (Seoul, Korea). CS was obtained from bovines and contained purified chondroitin sulfate sodium at a concentration of not less than 90%, calculated on a dry basis. Before starting the experiment, the rats were randomly assigned to a normal control group (NC group, n = 10), an OA control group (OAC group, n = 10), and CS treatment groups receiving 300 mg/kg (300 group, n = 10), 600 mg/kg (600 group, n = 10), and 900 mg/kg (900 group, n = 10). In the NC and OAC groups, animals were orally administered distilled water, and CS treatment groups were orally administered the appropriate dose for 6 weeks.

Osteoarthritis induction

After 2 weeks of oral administration, a single injection of 50 μL of sterile 0.9% saline containing 3 mg/kg MIA (Sigma-Aldrich, St. Louis, MO, USA) was injected into the intra-articular space of the right knee using a 31-gauge ultrafine insulin syringe (Becton Dickinson, Franklin Lakes, NJ, USA) inserted to a depth of approximately 2-3 mm. This step was performed under anesthesia induced by an intraperitoneal injection of ketamine and xylazine [23]. The NC group received an equivalent volume of saline instead of MIA. After the injection of MIA, the rats with MIA-induced OA received distilled water or the same doses of CS for 4 weeks. The treatment groups received daily oral doses of CS for a total of 6 weeks. The administration volumes were 2 mL for all experimental groups.

Sample collection

After 6 weeks of treatment, all rats were euthanized after being anesthetized by an intraperitoneal injection of ketamine and xylazine. Serum and knee cartilage were separated and frozen at -80°C until use.

ELISA

The levels of matrix metalloproteinases (MMPs; MMP-2, MMP-3, MMP-9, and MMP-13) and TIMP-1 in the serum were measured using Enzyme-linked Immunosorbent Assay (ELISA) Kits (Elabscience, Houston, Texas, USA). Each serum sample and standard was reacted at 37°C for 90 minutes. After the reaction, washing was performed three times using washing buffer, and then 100 μL of detection antibody was added, followed by reaction at 37°C for 60 minutes. After washing, 100 μL of HRP conjugate was added and reacted at 37°C for 30 minutes. After washing, 90 μL of substrate reagent was added and reacted at 37°C for 15 minutes. Finally, 50 μL of stop solution was added and absorbance at 450 nm was measured using a SpectraMax 400 (Molecular Devices, San Jose, CA, USA). Values were obtained based on the standard curve.

Table 1. Primer sequences

Gene name	Primer sequences
GAPDH	5'-AGTGCCAGCCTCGTCTCATA-3' (sense)
	5'-ACCATGTAGTTGAGGTCAAT-3' (antisense)
MMP-2	5'-TCCCGAGATCTGCAAGCAAG-3' (sense)
	5'-AGAATGTGGCCACCAGCAAG-3' (antisense)
MMP-3	5'-AAGATCCATGGAAGGCGTCG-3' (sense)
	5'-TCAGTGCGCCAAGTTTCAGA-3' (antisense)
MMP-9	5'-GATCCCCAGAGCGTTACTCG-3' (sense)
	5'-GTTGTGGAAACTCACACGCC-3' (antisense)
MMP-13	5'-TGCTGCATACGAGCATCCAT-3' (sense)
	5'-TGTCCTCAAAGTGAACCGCA-3' (antisense)
TIMP-1	5'-CTGCAACTCGGACCTGGTTA-3' (sense)
	5'-CAGCGTCGAATCCTTTGAGC-3' (antisense)

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitor of metalloproteinases.

Quantitative real-time polymerase chain reaction analysis

Samples for RNA extraction were obtained from the cartilage tissue of rats by cryogenic grinding, and total RNA was extracted using the Easy-spin (DNA free) Total RNA Extraction Kit (Intron Biotechnology Inc., Seoul, Korea) according to the manufacturer's instructions. A total of 1 μg of total RNA was reverse transcribed in a volume of 20 μL using oligo (dT) primers, with the enzyme and buffer supplied in the AccuPower® CycleScript RT PreMix (Bioneer, Daejeon, Korea). Quantitative real-time polymerase chain reactions (PCRs) were performed using a Rotor-Gene Q device (Qiagen, Hilden, Germany); the primers are shown in **Table 1**. The QuantiTect SYBR® Green PCR Kit (Qiagen) was used for real-time PCR. The final volume of the reaction mixture was 25 μL, including 2 μL of cDNA template, 12.5 μL of Master Mix, 1 μL of each primer (10 μM stock solution), and 8.5 μL of sterile distilled water. The thermal cycling profile consisted of a pre-incubation step at 95°C for 10 minutes, followed by 40 cycles at 95°C (15 seconds) and 60°C (60 seconds). Relative quantitative evaluations of *MMP-2, MMP-3, MMP-9, MMP-13*, and *TIMP-1* were performed using a threshold value of 25 cycles.

Histological analysis

Tissue specimens from the right knee joints were removed, fixed in 10% formalin, decalcification, embedded in paraffin, and serially sectioned at a thickness of 7 μm. The tissue sections were then stained with hematoxylin and eosin (H&E). Histological changes were examined by light microscopy (Axioskop 40; Carl Zeiss, Oberkochen, Germany) and images were obtained (Axiocam ICc1; Carl Zeiss). Cartilage degeneration of the medial tibial plateau was evaluated using the Osteoarthritis Research Society International (OARSI) score [24]. This scoring system measures the medial tibia plateau on a scale of 0-5 (0 = no degeneration, 1 = mild degeneration at the surface, 2 = slightly extended degeneration in the upper center, 3 = moderate degeneration in the median area, 4 = extended deep degeneration, and 5 = severe degeneration). All sections were observed and evaluated by three authors.

Morphological analysis

The microarchitecture of knee joints at 0.6-2.1 mm from the growth plate of the distal femur was scanned using a micro-computed tomography (micro-CT) system (Sky-Scan 1076; SkyScan, Aartselaar, Belgium). The X-ray source was set to a voltage of 50 kV and a current of 200 μA, and the beam was filtered using a 0.5-mm aluminum filter. The scanning angular rotation was 180° with an angular step of 0.5° [25]. The voxel size was fixed at 8.9 μm. The morphometric indices of the cartilage region were determined using CTAn (SkyScan 1076; SkyScan). All sections were observed and evaluated by three authors.

Data analysis

All results are reported as mean ± standard deviation. Results were analyzed by paired *t*-tests for two-group comparisons and by one-way ANOVA for comparisons among more than two groups using SPSS, Version 24.0 (IBM, Armonk, NY, USA). Values of $p < 0.05$, 0.01, and 0.001 were considered significant.

Results

Effects of CS on biomarker levels in the serum

The NC and OAC groups exhibited significant differences ($p < 0.01$) in the levels of MMPs and TIMP-1. After treatment with CS, the 600 and 900 groups showed significant decreases ($p < 0.05$) in levels of MMP-2 (**Figure 1A**),

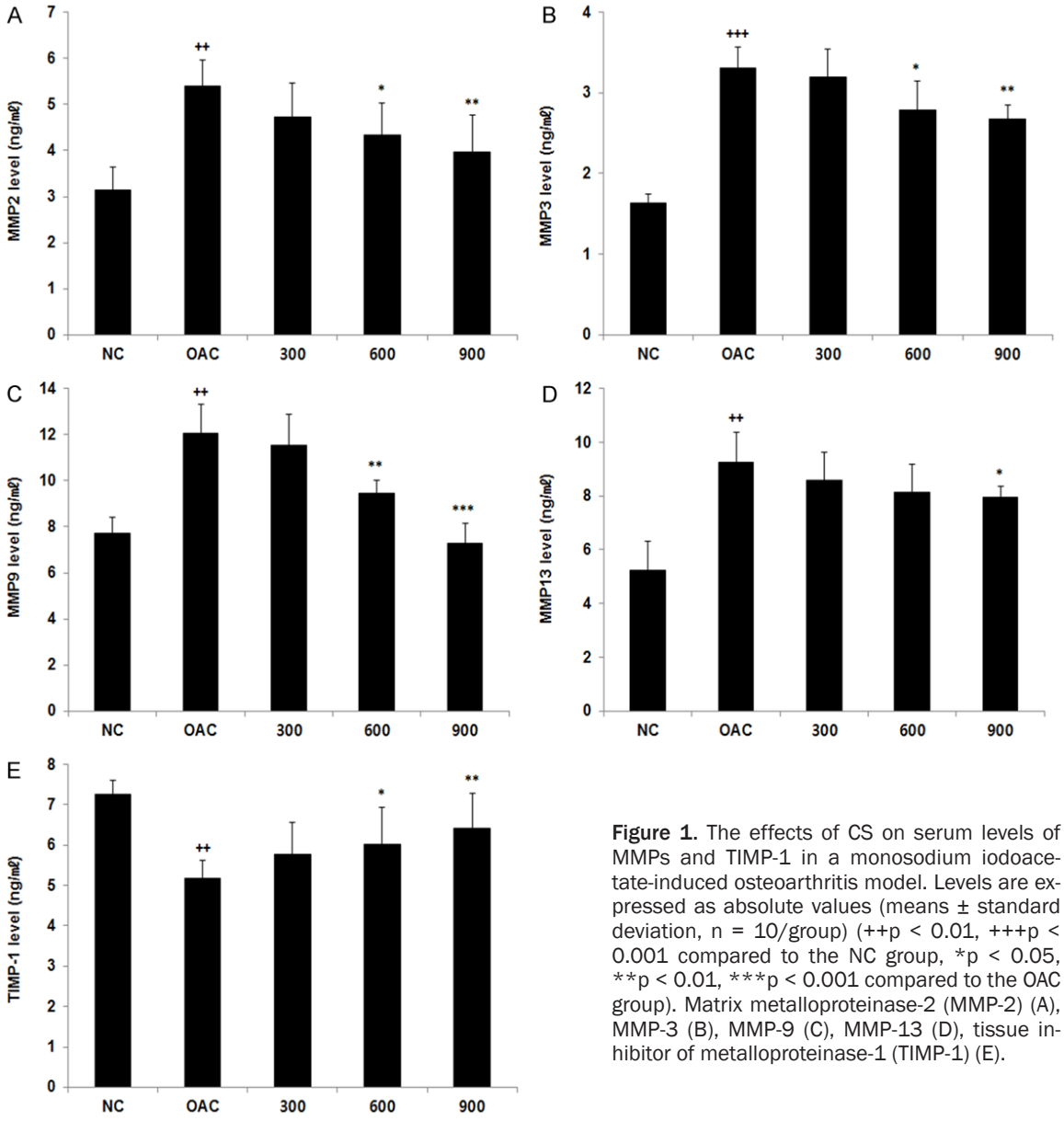

Figure 1. The effects of CS on serum levels of MMPs and TIMP-1 in a monosodium iodoacetate-induced osteoarthritis model. Levels are expressed as absolute values (means ± standard deviation, n = 10/group) (++p < 0.01, +++p < 0.001 compared to the NC group, *p < 0.05, **p < 0.01, ***p < 0.001 compared to the OAC group). Matrix metalloproteinase-2 (MMP-2) (A), MMP-3 (B), MMP-9 (C), MMP-13 (D), tissue inhibitor of metalloproteinase-1 (TIMP-1) (E).

MMP-3 (**Figure 1B**), and MMP-9 (**Figure 1C**) and a significant increase (p < 0.05 and 0.01) in TIMP-1 (**Figure 1E**). However, only MMP-13 (**Figure 1D**) exhibited a significant decrease (p < 0.05) in the 900 group.

Effects of CS on biomarker expression at the mRNA level

The OAC group exhibited significantly higher mRNA expression levels of *MMP-2, MMP-3, MMP-9*, and *MMP-13* (p < 0.001), and significantly lower *TIMP-1* levels (p < 0.01) compared to those in the NC group (**Figure 2**). After treatment with CS, the 600 and 900 groups showed significant down-regulation (p < 0.05) of *MMP-2* (**Figure 2A**), *MMP-3* (**Figure 2B**), *MMP-9* (**Figure 2C**), and *MMP-13* (**Figure 2D**). Additionally, *TIMP-1* (**Figure 2E**) mRNA expression was significantly up-regulated (p < 0.05).

Effects of CS on OA histopathology

Compared to the NC group, the OAC and CS groups showed decreases in CS between the cartilage (yellow circle in **Figure 3**) and decreased cartilage between the femur and tibia. However, in the CS group, the amount of CS was maintained in a dose-dependent manner and CS levels around the joints were greater

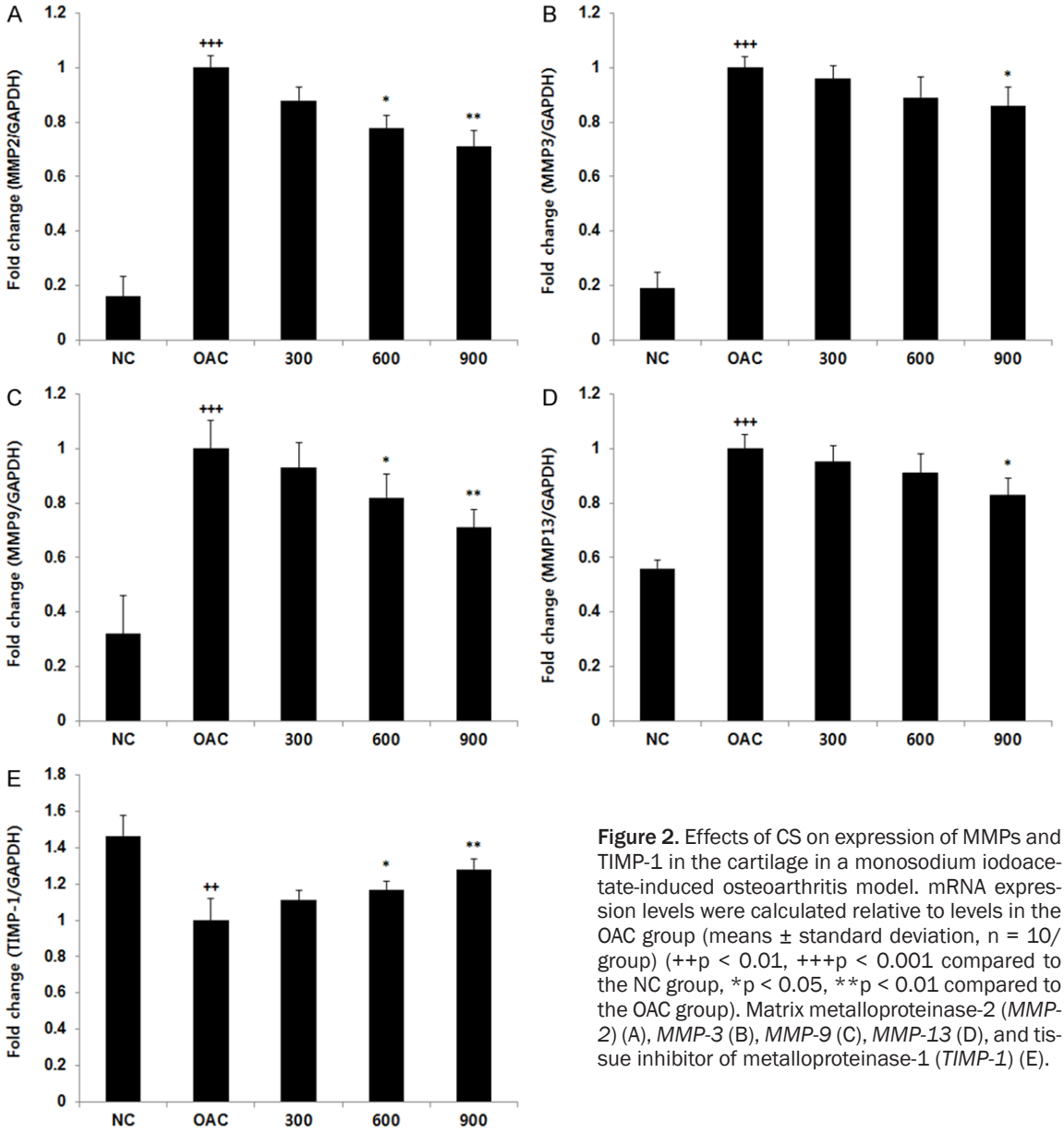

Figure 2. Effects of CS on expression of MMPs and TIMP-1 in the cartilage in a monosodium iodoacetate-induced osteoarthritis model. mRNA expression levels were calculated relative to levels in the OAC group (means ± standard deviation, n = 10/group) (++p < 0.01, +++p < 0.001 compared to the NC group, *p < 0.05, **p < 0.01 compared to the OAC group). Matrix metalloproteinase-2 (*MMP-2*) (A), *MMP-3* (B), *MMP-9* (C), *MMP-13* (D), and tissue inhibitor of metalloproteinase-1 (*TIMP-1*) (E).

than those in the OAC group (**Figure 3A**). Based on Safranin-O staining, proteoglycan (yellow arrow in **Figure 3B**) staining in the periphery of the cartilage and synovial membrane in the OAC group and CS group were not distributed evenly compared to that in the NC group. In the CS group, proteoglycan levels around the cartilage and synovial membrane were higher than those in the OAC group, which showed a lack of proteoglycans in these regions (**Figure 3B**). Additionally, the OARSI histological score was significantly higher in the OAC group than in the NC group ($p < 0.001$). After treatment with CS, the 600 and 900 groups showed significantly lower OARSI scores ($p < 0.001$) compared to that in the OAC group (**Figure 3C**).

Effects of CS on femur morphology and articular cartilage volume

Compared to the NC group, the OAC group and the CS group showed marked decreases in the cartilage volume (purple area in the yellow circle in **Figure 4**) and severe bone destruction in the lower femur region. There were no significant differences in bone destruction between the CS group and the OAC group, but the cartilage volume was better preserved in the CS group.

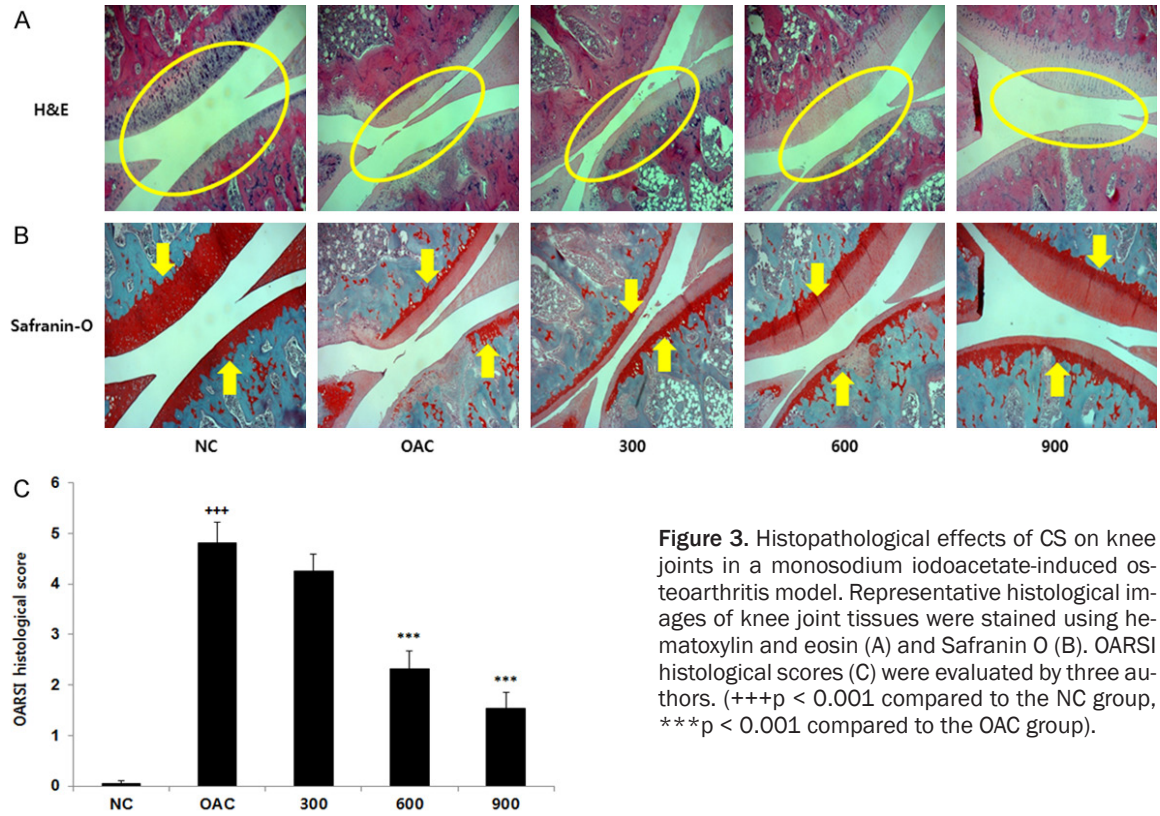

Figure 3. Histopathological effects of CS on knee joints in a monosodium iodoacetate-induced osteoarthritis model. Representative histological images of knee joint tissues were stained using hematoxylin and eosin (A) and Safranin O (B). OARSI histological scores (C) were evaluated by three authors. (+++$p < 0.001$ compared to the NC group, ***$p < 0.001$ compared to the OAC group).

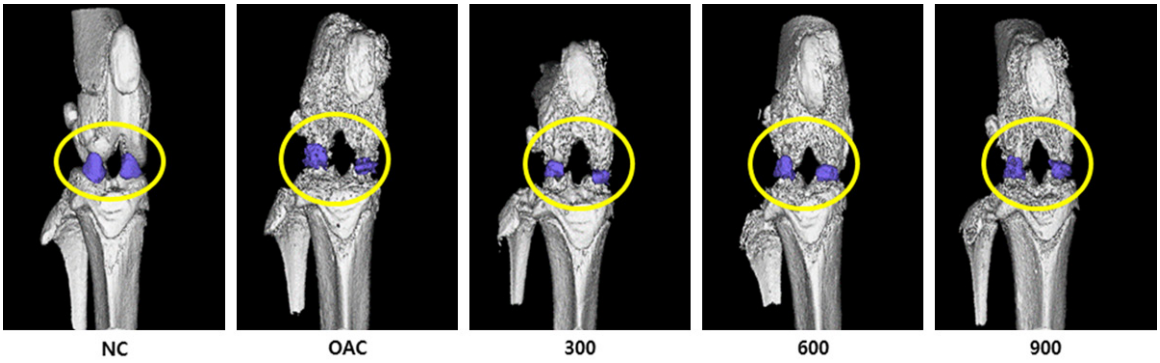

Figure 4. Effects of CS on femur bone architecture and cartilage volume in a monosodium iodoacetate-induced osteoarthritis model. Representative morphological images of the knee joint tissues were obtained by three-dimension micro-computed tomography.

Discussion

OA is one of the most common joint diseases in adults. It is accompanied by a gradual loss of joint cartilage and secondary changes and symptoms associated with local joints. It is characterized mainly by local degenerative changes of the articular cartilage, hypertrophy of the cartilage, excessive bone formation of the surrounding osteochondral region, and deformation of the joint, resulting in repeated pain, arthroscopic instinct, and gradual movement disorders [26, 27].

Currently, OA is treated with surgery or medication, with the aim of relieving pain and symptoms. Analgesics, nonsteroidal anti-inflammatory drugs, and symptomatic slow-acting drugs in OA are prescribed [28], but their use has been limited owing to their poor therapeutic efficacy and side effects. Therefore, better materials for OA treatment are needed.

CS occurs naturally in the extracellular matrix of the articular cartilage and is composed of *N*-acetylgalactosamine and glucuronic acid, which are glycosaminoglycans composed of long unbranched polysaccharides with a repeating disaccharide unit [29]. They stimulate chondrocytes to produce proteoglycans and collagen, regenerate cartilage, and prevent cartilage destruction [30, 31]. In this study, the efficacy of CS for the prevention and recovery of cartilage destruction, which is a factor in OA, was evaluated.

MMPs are the main mediators of cartilage destruction. They can degrade all components of the extracellular matrix. Damaged chondrocytes in OA produce pro-inflammatory cytokines, such as IL-1β and TNF-α, creating an inflammatory environment, and these cytokines increase the synthesis of MMPs [32, 33]. In this study, oral administration of CS reduced the levels of MMPs by greater than 15% compared to levels in the OAC group (**Figure 1A-D**), and mRNA expression levels of MMPs were also down-regulated by greater than 15% compared to levels in the OAC group (**Figure 1E**).

TIMP-1 can block the activity of all MMPs, except membrane type 1 MMP [9]. In this study, the oral administration of CS reduced the levels of TIMP-1 by greater than 24% compared to levels in the OAC group (**Figure 2A-D**), and the mRNA expression levels of *TIMP-1* were also up-regulated by greater than 28% compared to those in the OAC group (**Figure 2E**).

Therefore, MMPs and TIMP-1 are potential targets for OA therapy because they play important roles in cartilage destruction [34]. CS seems to have a protective effect against cartilage destruction caused by OA. It has an inhibitory effect on MMP activity increased by OA. Based on histological and morphological analyses, cartilage deformation was greater in the OAC group than in the NC group. The CS group showed less deformation of cartilage than that in the OAC group. In addition, Safranin-O staining showed that the OAC group had more proteoglycan layer destruction than that of the NC group. The CS group showed less destruction of the proteoglycan layer than that of the OAC group. Finally, micro-CT-arthrography was used to analyze the knee joint cartilage volume. The CS group had an increased cartilage volume compared to that of the OAC group.

In this study, MMPs and TIMP-1 were examined at the protein and mRNA levels in the blood and injured cartilage tissue. CS protected damaged cartilage cells by regulating biomarkers associated with OA. These results provided additional evidence for the clinical treatment of OA using CS.

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References

[1] Wittenauer R, Smith L and Aden K. Background paper 6.12 osteoarthritis. World Health Organisation. 2013.

[2] Neogi T and Zhang Y. Epidemiology of osteoarthritis. Rheum Dis Clin 2013; 39: 1-19.

[3] Yoshimura N, Muraki S, Oka H, Mabuchi A, En-Yo Y, Yoshida M, Saika A, Yoshida H, Suzuki T, Yamamoto S, Ishibashi H, Kawaguchi H, Nakamura K and Akune T. Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the research on osteoarthritis/osteoporosis against disability study. J Bone Miner Metab 2009; 27: 620-628.

[4] Konttinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, Kinne RW, Santavirta S, Sorsa T, Lopez-Otin C and Takagi M. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis 1999; 58: 691-697.

[5] Yoshihara Y, Nakamura H, Obata K, Yamada H, Hayakawa T, Fujikawa K and Okada Y. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis 2000; 59: 455-461.

[6] Sledge C, Reddi AH, Walsh D and Blake D. Biology of the normal joint. Kelley's textbook of rheumatology. Philadelphia, Elsevier, 2001, pp. 1-26.

[7] Lewis EJ, Bishop J, Bottomley KM, Bradshaw D, Brewster M, Broadhurst MJ, Brown PA, Budd JM, Elliott L, Greenham AK, Johnson WH, Nixon JS, Rose F, Sutton B and Wilson K. Ro 32-3555, an orally active collagenase inhibitor,

prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 1997; 121: 540-546.
[8] Baker AH, Edwards DR and Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci 2002; 115: 3719-3727.
[9] Brew K, Dinakarpandian D and Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function1. Biochim Biophys Acta Protein Struct Mol Enzymol 2000; 1477: 267-283.
[10] Jomphe C, Gabriac M, Hale TM, Héroux L, Trudeau LÉ, Deblois D, Montell E, Vergés J and Du Souich P. Chondroitin sulfate inhibits the nuclear translocation of nuclear factor-κB in interleukin-1β-stimulated chondrocytes. Basic Clin Pharmacol Toxicol 2008; 102: 59-65.
[11] du Souich P, Garcia AG, Verges J and Montell E. Immunomodulatory and anti-inflammatory effects of chondroitin sulphate. J Cell Mol Med 2009; 13: 1451-1463.
[12] Martel-Pelletier J, Kwan Tat S and Pelletier JP. Effects of chondroitin sulfate in the pathophysiology of the osteoarthritic joint: a narrative review. Osteoarthritis Cartilage 2010; 18: 7-11.
[13] Nerucci F, Fioravanti A, Cicero M, Collodel G and Marcolongo R. Effects of chondroitin sulfate and interleukin-1β on human chondrocyte cultures exposed to pressurization: a biochemical and morphological study. Osteoarthritis Cartilage 2000; 8: 279-287.
[14] Pecchi E, Priam S, Mladenovic Z, Gosset M, Saurel AS, Aguilar L, Berenbaum F and Jacques C. A potential role of chondroitin sulfate on bone in osteoarthritis: inhibition of prostaglandin E2 and matrix metalloproteinases synthesis in interleukin-1β-stimulated osteoblasts. Osteoarthritis Cartilage 2012; 20: 127-135.
[15] Largo R, Roman-Blas JA, Moreno-Rubio J, Sanchez-Pernaute O, Martinez-Calatrava MJ, Castaneda S and Herrero-Beaumont G. Chondroitin sulfate improves synovitis in rabbits with chronic antigen-induced arthritis. Osteoarthritis Cartilage 2010; 18: 17-23.
[16] Bauerova K, Ponist S, Kuncirova V, Mihalova D, Paulovicova E and Volpi N. Chondroitin sulfate effect on induced arthritis in rats. Osteoarthritis Cartilage 2011; 19: 1373-1379.
[17] Uebelhart D, Thonar EJ, Delmas PD, Chantraine A and Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. Osteoarthritis Cartilage 1998; 6: 39-46.
[18] Möller I, Pérez M, Monfort J, Benito P, Cuevas J, Perna C, Domenech G, Herrero M, Montell E and Verges J. Effectiveness of chondroitin sulphate in patients with concomitant knee osteoarthritis and psoriasis: a randomized, double-blind, placebo-controlled study. Osteoarthritis Cartilage 2010; 18: 32-40.
[19] Wildi LM, Raynauld JP, Martel-Pelletier J, Beaulieu A, Bessette L, Morin F, Abram F, Dorais M, Pelletier JP. Chondroitin sulphate reduces both cartilage volume loss and bone marrow lesions in knee osteoarthritis patients starting as early as 6 months after initiation of therapy: a randomised, double-blind, placebo-controlled pilot study using MRI. Ann Rheum Dis 2011; 70: 982-989.
[20] Kobayashi K, Imaizumi R, Sumichika H, Tanaka H, Goda M, Fukunari A and Komatsu H. Sodium iodoacetate-induced experimental osteoarthritis and associated pain model in rats. J Vet Med Sci 2003; 65: 1195-1199.
[21] Marker CL and Pomonis JD. The monosodium iodoacetate model of osteoarthritis pain in the rat. Methods Mol Bio 2012: 239-248.
[22] Fernihough J, Gentry C, Malcangio M, Fox A, Rediske J, Pellas T, Kidd B, Bevan S and Winter J. Pain related behaviour in two models of osteoarthritis in the rat knee. Pain 2004; 112: 83-93.
[23] Vonsy JL, Ghandehari J and Dickenson AH. Differential analgesic effects of morphine and gabapentin on behavioural measures of pain and disability in a model of osteoarthritis pain in rats. Eur J Pain 2009; 13: 786-793.
[24] Gerwin N, Bendele AM, Glasson S and Carlson CS. The OARSI histopathology initiative-recommendations for histological assessments of osteoarthritis in the rat. Osteoarthritis Cartilage. 2010; 18: 24-34.
[25] Chung HJ, Cho L, Shin JS, Lee J, Ha IH, Park HJ and Lee SK. Effects of JSOG-6 on protection against bone loss in ovariectomized mice through regulation of osteoblast differentiation and osteoclast formation. BMC Complement Altern Med 2014; 14: 184.
[26] Zamli Z, Adams MA, Tarlton JF and Sharif M. Increased chondrocyte apoptosis is associated with progression of osteoarthritis in spontaneous Guinea pig models of the disease. Int J Mol Sci 2013; 14: 17729-17743.
[27] Han G, Chen Q, Liu F, Cui Z, Shao H, Liu F, Ma A, Liao J, Guo B, Guo Y, Wang F, Ling P and Mei X. Low molecular weight xanthan gum for treating osteoarthritis. Carbohydr Polym 2017; 164: 386-395.
[28] Lequesne M, Brandt K, Bellamy N, Moskowitz R, Menkes CJ, Pelletier JP and Altman R. Guidelines for testing slow acting drugs in osteoarthritis. J Rheumatol 1994; 41: 65-71.
[29] Hardingham TE and Fosang AJ. Proteoglycans: many forms and many functions. FASEB J 1992; 6: 861-870.
[30] Jerosch J. Effects of glucosamine and chondroitin sulfate on cartilage metabolism in OA: outlook on other nutrient partners especially omega-3 fatty acids. Int J Rheumatol 2011; 2011: 969012.

[31] Henrotin Y, Mathy M, Sanchez C and Lambert C. Chondroitin sulfate in the treatment of osteoarthritis: from in vitro studies to clinical recommendations. Ther Adv Musculoskelet Dis 2010; 2: 335-348.

[32] Caron JP, Fernandes JC, Martel-Pelletier J, Tardif G, Mineau F, Geng C and Pelletier JP. Chondroprotective effect of intraarticular injections of interleukin-1 receptor antagonist in experimental osteoarthritis. Suppression of collagenase-1 expression. Arthritis Rheum 1996; 39: 1535-1544.

[33] Van De Loo FA, Joosten LA, Van Lent PL, Arntz OJ and Van Den Berg WB. Role of interleukin-1, tumor necrosis factor α, and interleukin-6 in cartilage proteoglycan metabolism and destruction effect of in situ blocking in murine antigen-and zymosan-induced arthritis. Arthritis Rheum 1995; 38: 164-172.

[34] Will H, Atkinson SJ, Butler GS, Smith B and Murphy G. The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of progelatinase A and initiates autoproteolytic activation. Regulation by TIMP-2 and TIMP-3. J Biol Chem 1996; 271: 17119-17123.