대한침구학회지 제22권 제2호(2005년 4월)
The Journal of Korean Acupuncture & Moxibustion Society

원 저

Effects of Aralia cordata Thunb. on Proteoglycan Release, Type II Collagen Degradation and Matrix Metalloproteinase Activity in Rabbit Articular Cartilage Explants

Baek Yong-hyeon[*], Seo Byung-kwan[*], Lee Jae-dong[*], Huh Jeong-eun[**], Yang Ha-ru[**], Cho Eun-mi[**], Choi Do-young[*], Kim Deog-yoon[#], Cho Yoon-je[##], Kim Kang-il[##] and Park Dong-suk[*]

[*]Dept. of Acupuncture & Moxibustion, College of Oriental Medicine, KyungHee University
[**]Oriental Medicine Research Center for Bone & Joint Disease, KyungHee University
[#]Dept. of Nuclear Medicine and [##]Dept. of Orthopedic Surgery, College of Medicine, KyungHee University

Abstract

Background & Objective : Articular cartilage is a potential target for drugs designed to inhibit the activity of matrix metalloproteinases (MMPs) to stop or slow the destruction of the proteoglycan and collagen in the cartilage extracellular matrix. The purpose of this study was to investigate the effects of Aralia cordata Thunb. in inhibiting the release of glycosaminoglycan (GAG), the degradation of collagen, and MMP activity in rabbit articular cartilage explants.

Methods : The cartilage-protective effects of Aralia cordata Thunb. were evaluated by using glycosaminoglycan degradation assay, collagen degradation assay, colorimetric analysis of MMP activity, measurement of lactate dehydrogenase activity and histological analysis in rabbit cartilage explants culture.

Results : Interleukin-1a (IL-1a) rapidly induced GAG, but collagen was much less readily released from cartilage explants. Aralia cordata Thunb. significantly inhibited GAG and collagen release in a concentration-dependent manner. Aralia cordata Thunb. dose-dependently inhibited MMP-3 and MMP-13 expression and activities from IL-1a-treated cartilage explants cultures when tested at concentrations ranging from 0.02 to 0.2 mg/ml. Aralia cordata Thunb. had no harmful effect on chondrocytes viability or cartilage morphology in cartilage explants. Histological analysis indicated that Aralia cordata Thunb. reduced the degradation of the cartilage matrix compared with that of IL-1a-treated cartilage explants.

· **Acceptance** : 2005. 3. 10. · **Adjustment** : 2005. 3. 12. · **Adoption** : 2005. 3. 17.
· **Corresponding author** : Park Dong-suk, Dept. of Acupuncture & Moxibustion, College of Oriental Medicine, KyungHee University, Seoul, Korea 130-702
Tel. 82-2-958-9206 Fax. 82-2-958-9043 E-mail : dspark49@yahoo.co.kr

Conclusion : These results indicate that Aralia cordata Thunb. inhibits the degradation of proteoglycan and collagen through the downregulation of MMP-3 and MMP-13 activities without affecting the viability or morphology of IL-1a-stimulated rabbit articular cartilage explants.

Key words : Aralia cordata Thunb. articular cartilage, proteoglycan, collagen, matrix metalloproteinase

Ⅰ. Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive loss of articular cartilage, subchondral bond remodeling, spur formation,synovial inflammation, and in particular, the degradation of proteoglycan and collagen. The integrity of these macromolecules is vital to cartilage and joint function[1)].

Proteoglycan is a component of the articular cartilage extracellular matrix, providing it with many of its characteristic physicochemical properties[2)]. The carbohydrate component of aggrecan, which constitutes at least 90% of its molecular mass, consists of many long keratin sulfate, chondroitin sulfate, and glycosaminoglycan (GAG) chains covalently linked to a core protein[3)]. Thus, the importance of these proteoglycans is clear. However, neither their specific role nor the mechanisms regulating their synthesis are fully understood.

Collagen is another component of the articular cartilage, which consists primarily of type Ⅱ collagen[4)]. Itplays a role in maintaining the integrity of the cartilage matrix and allows proteoglycan to be held in the matrix[5)]. There iscircumstantial in vitro and in vivo evidence indicating a significant role for matrix metalloproteinases (MMPs) in cartilage destruction in arthritis[6)].

MMPs can be classified into four subgroups:collagenases (MMP-1, -8, -13), stromyelins (MMP-3, -10, -11), gelatinases (MMP-2, -9), and membrane-type MMPs[7)]. MMP-3 is capable of cleaving the aggrecan core protein, as well as type Ⅱ collagen (in the amino-terminal telopeptide) in vitro, but it is not clear if it is involved in the degradation of these proteins in cartilage[8-9)]. MMP-13 is the most efficient collagenase against type Ⅱ collagen, suggesting it hasan important role in cartilage collagen turnover[10-11)].

The root of Aralia cordata Thunb. has been used in the treatment of arthritis and low back pain. It has been reported that several compounds of Aralia cordata Thunb. inhibited COX-2 dependent PGE2 generation[12)] and were significantly effective regarding analgesics, hypothermia, duration of pentobarbital-induced anesthesia[13)]. But there is no report related with the cartilage-protective effects of Aralia cordata Thunb.

The present study there investigated the cartilage-protective effects of Aralia cordata Thunb. on rabbit articular cartilage. We also characterized the mechanism of these protective effects.

Ⅱ. Materials and methods

1. Preparation of Aralia cordata Thunb.

The root of Aralia cordata Thunb. was extracted at room temperature in 70% (v/v)

ethanolwater for 24 h. The extract was then filtered and concentrated under low pressure using a vacuum rotary evaporator (Eyela, Japan). The remaining residue was lyophilized in a freeze-dryer, and stored at 20 C. The powder was dissolved in dimethyl sulfoxide (DMSO) and diluted with Dulbecco's modified Eagle's medium (DMEM) to final concentrations of total extract ranging from 0.02 to 0.2 mg/ml.

2. Cartilage explants culture

Articular cartilages were obtained from the joints of five-week-old rabbits (Samtako Biokorea Co., Korea). In brief, the articular surfaces were surgically exposed under sterile conditions; approximately 200-220 mg of articular surface per joint was removed and steeped in complete medium (DMEM supplemented with heat-inactivated 5% fetal bovine serum [FBS] and 100 unit/ml of penicillinstreptomycin [Gibco BRL, Maryland, USA]). The samples were then rinsed several times with complete medium and incubated for 12 days at 37 ℃ in a humidified CO_2/95% air incubator to stabilize them. The complete medium was replaced with basal medium (DMEM supplemented with heat-inactivated 1% FBS, 10 mM HEPES, and 100 unit/ml penicillinstreptomycin). Approximately 30 mg of cartilage pieces were placed in 48-well plates and treated with various concentrations of extract of Aralia cordata Thunb. After 1 h of pretreatment, 5 ng/ml IL-1? (R&D Systems, Minneapolis, USA) was added to the culture media, which were then incubated at 37 C for a further 3 days. The supernatants were harvested and replaced with fresh mediacontaining test reagents. These were incubated for a further 25 days, and 3, 7, 14, and 28 days supernatant were collected and stored at 20 C until assayed.

3. Glycosaminoglycan degradation assay

Glycosaminoglycan levels in the culture medium were determined by the amount of polyanionic material reacting with 1,9-dimethylmethylene blue, using shark chondroitin sulfate as thestandard. Samples were examined spectrophotometrically at 540 nm (Spectramax, Molecular Devices, Sunnyvale, CA, USA). The percentage recovery was calculated from the peak height of the sample relative to that of the standard.

4. Collagen degradation assay

Type II collagen levels in the culture medium were determined using the Sircol Collagen Assay (Biocolor Ltd., Valley Business Center, Northern Ireland). Samples were reacted with Sirius red dye containing sulfonic acid for 30 min at room temperature. The reaction mixture measured optical density at 540 nm. The percentage of recovery was calculated from the peak height of the sample relative to that of the standard.

5. Colorimetric analysis of MMP activity

The levels of MMP activity in the conditioned media were evaluated using anenzyme-linked immunosorbent assay (ELISA) kit (Biomol Research Lab., Inc., PA, USA) according to the manufacturer's instructions. Briefly, MMP activity was measured using a thiopeptolide as a colorimetric substrate (Ac-Pro-Leu-Gly-[2-mercapto-4-methyl-pentanoyl]-Leu-Gly-OC_2H_5), which is cleaved by stromyelin-1/MMP-3 and collagenase-3/MMP-13. To assess the proteolytic activity, 25

$\mu\ell$ of each sample was pipetted into 96 well plates together with each enzyme, buffer, and substrate. After 1 h of incubation at 37 ℃, the samples were measured at 405 nm. For each sample, MMP-3 and MMP-13 activities were measured as a percentage of the MMPs in that culture well.

6. Measurement of lactate dehydrogenase activity

As anindicator of cell viability, the cytoplasmic enzyme lactate dehydrogenase (LDH) was measured in the culture medium. An optimized LDH test (Promega Corp., Madison, WI, USA) was used to quantify LDH activity in the medium of the cartilage explants cultures.

7. Histology

Cartilage explants pieces were fixed in 10% neutral formalin, dehydrated with graded ethanol, embedded in paraffin, and sectioned into 4 m slices. Sectioned tissues were stained with hematoxylin and eosin (H&E) for light microscopic examination. To detect proteoglycan and collagen in the cartilage, duplicate sections were stained with Safranin O and Masson'sTrichrome. The number of chondrocytes was measured in three identically treated cartilage explants using a 200x lens. Pathologist with no prior knowledge of the test reagents examined the stained slides.

8. Statistical analysis

The results were expressed as means ±S.D. calculated from the specified numbers of determinations. Statistically significant differences relative to the untreated control group were calculated by Student's one-tailed paired t test. Differences with p values < 0.05 were deemed statistically significant.

Ⅲ. Results

1. Dose response and time course of IL-1a-induced cartilage degradation

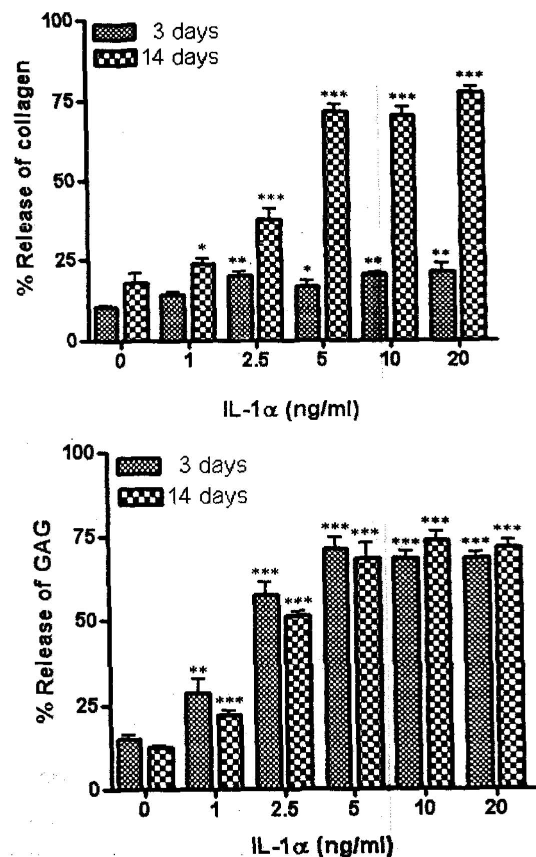

Fig. 1. Dose response and time course of IL-1a-induced rabbit cartilage explants cultures. (A) GAG is shown as a percentage of the cumulative release into media treated with different concentrations of interleukin-1a (IL-1a) for 14 days in rabbit cartilage explants. Media were replenished with fresh IL-1a once every 3 days. (B) Collagen degradation is shown as a percentage of the cumulative release into the media of rabbit cartilage explants. Bars show the mean ±SD of three experiments.

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the respective controls.

In preliminary experiments to optimize the conditions with which to induceproteoglycan and collagen degradation, rabbit articular cartilage was cultured with 1, 2.5, 5, 10, or 20 ng/ml IL-1a for 14 days. These effects were dose-dependent and 5 ng/ml IL-1a was required to consistently achievethe maximal response. In experimental cultures of rabbit cartilage treated with 5 ng/ml IL-1a, over 74% of GAG had been released from the tissue after 3 days of culture and about 75% after 14 days (Fig. 1A). In parallel experiments, cartilage explants were cultured with various concentrations of IL-1a for 14 days. There was little release of type II collagen from the cartilage at any concentration of IL-1a for 3 days, after which there was a marked increase in collagen release to about 75% by day 14 of culture (Fig. 1B).

2. Effect of Aralia cordata Thunb. on proteoglycan and collagen degradation

To study whether Aralia cordata Thunb. affects proteoglycan and collagen degradation in rabbit cartilage explants, rabbit cartilage explants were cultured in the presence of 5 ng/ml IL-1a for 28 days. Aralia cordata Thunb. consistently reduced the IL-1a-mediated GAG release into the culture medium until 14 days (Fig. 2). Norelease of collagen into the culture medium was observed from explants treated with IL-1a alone until 7 days. After 14 days, Aralia cordata Thunb. markedly reduced collagen degradation relative to that in the IL-1a-treated cultures, and significantly reduced until 28 days (Fig. 2). Moreover, Aralia cordata Thunb. dose-dependently reduced IL-1a-mediated GAG and collagen release intothe culture medium (Fig. 3). Aralia cordata Thunb. significantly reduced GAG and collagen release starting from a low concentration of 0.02 mg/ml, and almost totally inhibited it at a concentration of 0.2 mg/ml for 14 days. Rofecoxib, a selective cyclooxygenase (COX-2) inhibitor, did not inhibit GAG orcollagen degradation at a concentration of 30 mM. Diclofenac, a

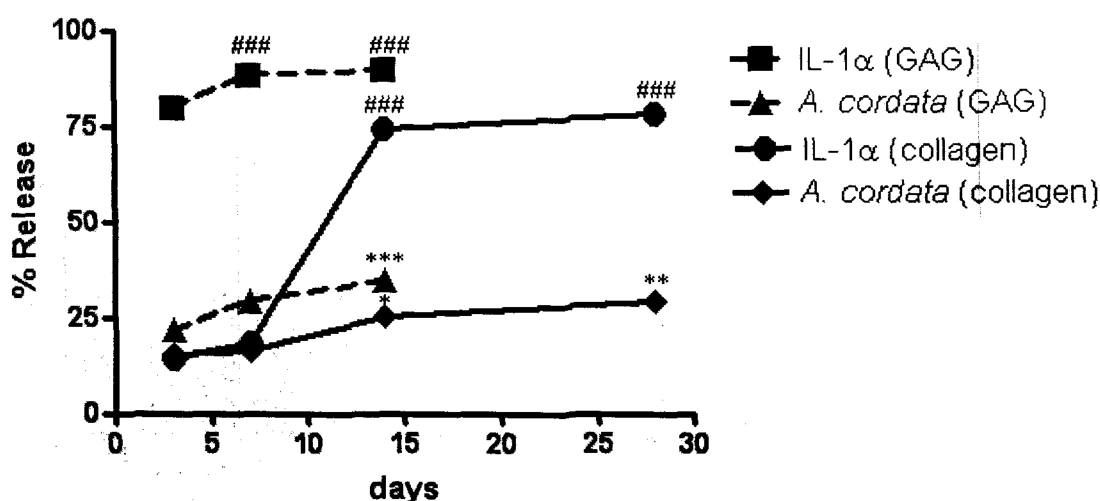

Fig. 2. Effect of Aralia cordata Thunb. on proteoglycan and collagen degradation in rabbit cartilage explants cultures over time. Cartilage was cultured in quadruplicate in 400 μl of medium only, with 5 ng/ml IL-1a, or with 5 ng/ml IL-1a + 0.1 mg/ml Aralia cordata Thunb. for 28 days. Media with or without IL-1a were replenished once every 3 days. GAG and collagen degradation are shown as the cumulative release into the medium, asa percentage of total GAG and collagen at different times in the culture. Bars show the mean ±SD of three experiments.

$p < 0.001$ compared with control, and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the respective controls (IL-1a).

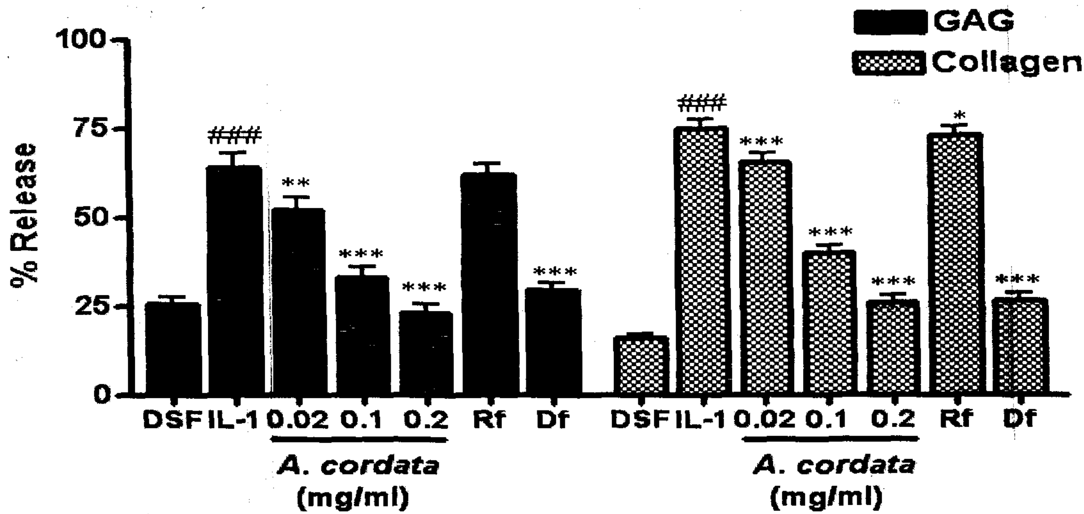

Fig. 3. Effect of Aralia cordata Thunb. on the dose response of proteoglycan and collagen degradation in rabbit cartilage explants cultures. Cartilage was cultured in quadruplicate in 400 $\mu\ell$ of medium only, with 5 ng/ml IL-1a, or with 5 ng/ml IL-1a plus different concentrations of Aralia cordata Thunb. for 14 days. The media were removed on 3 days and replaced as described above for a further 11 days. The levelsof GAG and collagen released into the medium on 14 days were measured and the results are expressed as a percentage of the total compound released. Bars show the mean ±SD of three experiments.

$p < 0.001$ compared with control, and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the respective control (IL-1a).

non-selective COX-2 inhibitor, effectively reducedGAG and collagen degradation at 30 mM (Fig. 3).

3. Effect of Aralia cordata Thunb. on MMP activity

We examined whether Aralia cordata Thunb. inhibited IL-1a-mediated MMP-3 and MMP-13 activities in the culture medium. We tested the

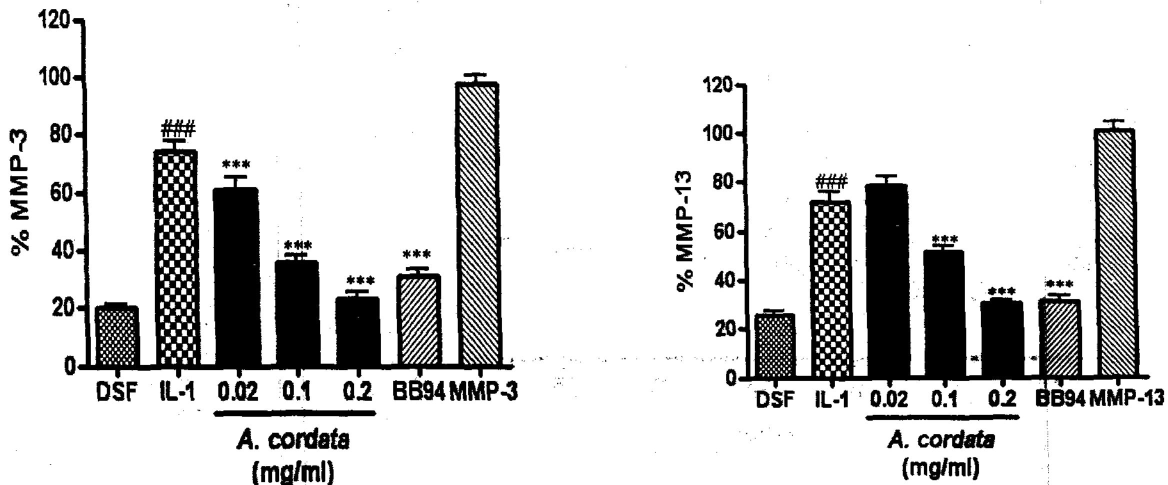

Fig. 4. Effect of Aralia cordata Thunb. on MMP activity in rabbit cartilage explants cultures. Cartilage was cultured in medium only, with 5 ng/ml IL-1a, with 5 ng/ml IL-1a plus different concentrations of Aralia cordata Thunb. or with 104 mM BB94, an MMP inhibitor, for 14 days. Cumulative MMP-3 and MMP-13 activities were analyzed with a colorimetric substrate assay. Bars show the mean ±SD of three experiments.

$p < 0.001$ compared with control, and ***$p < 0.001$ compared with respective control (IL-1a).

levels of MMP-3 and MMP-13 activity in the medium from cultures after 14 days with or without Aralia cordata Thunb.. MMP-3 and MMP-13 levels decreased dose-dependently in the culture media with Aralia cordata Thunb. at day 14 compared with the levels in IL-1a-treated cultures (Fig. 4). In Baralia cordata Thunb.-treated cultures, the levels of MMP-3 and MMP-13 activity were reduced more than in cultures treated with only BB94, an MMP inhibitor (Fig. 4).

4. Effect of Aralia cordata Thunb. on the viability of cartilage explants

We examined whether Aralia cordata Thunb. affects chondrocytes viability in cartilage explants cultures. We were unable to detect any LDH activity in the incubation medium of cultures treated with the drug or with IL-1a alone, indicating that neither IL-1a nor Aralia cordata Thunb.have cytotoxic effects on chondrocytes cartilage explants during 3, 7, or 14 days of culture (Fig. 5).

5. Effect of Aralia cordata Thunb. on the morphology of cartilage explants

We evaluated whether Aralia cordata Thunb. affects the structural integrity of cartilage or chondrocytes in IL-1a-induced cartilage explants cultures. Examination of sections of untreated cartilage explants revealed normal staining for proteoglycan and collagen with Safranin O and Masson's Trichrome (Fig. 6). In contrast, microscopic analysis of IL-1a-treated explants showed a reduction in the amounts of proteoglycan and collagen present. Aralia cordata Thunb.-treated cartilage showed more intense staining for proteoglycan and collagen compared with cartilage samples treated with IL-1a alone. The total number of chondrocytes increased 2.7-fold after 14 days in culture compared withthe number in cartilage treated with IL-1a alone. Neither IL-1a alone nor additional treatment with Aralia cordata Thunb.induced any pathological change in the cartilage explants.

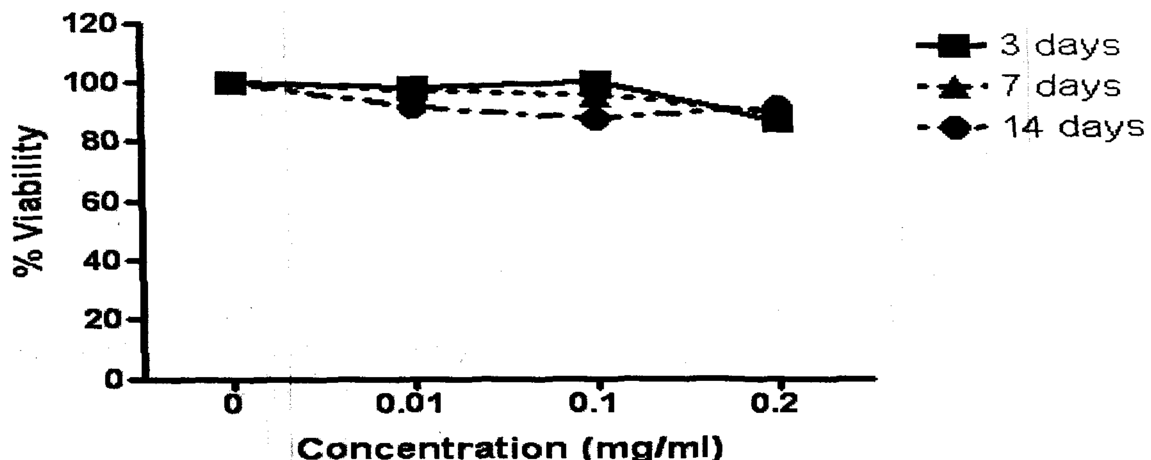

Fig. 5. Effect of Aralia cordata Thunb. on the viability of cartilage explants cultures. Cartilage was cultured in quadruplicate in 400 μl of medium only, with 5 ng/ml IL-1a, or with 5 ng/ml IL-1a plus different concentrations of Aralia cordata Thunb. for 14 days. Medium was removed on day 3 and replaced as described above for a further 11 days. The viability of chondrocytes in cartilage explants cultureswas measured on 3, 7, and 14 days and the results are expressed as a percentage of the total LDH released. Bars show the mean ±SD of three experiments.

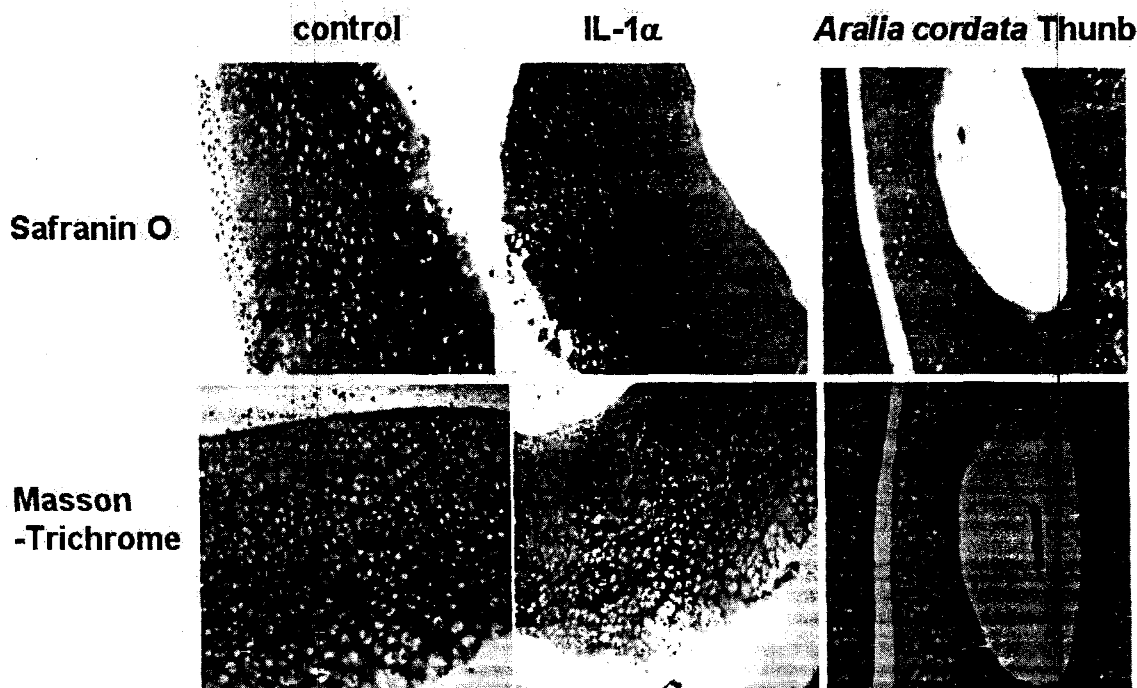

Fig. 6. Histochemical analysis of proteoglycan and collagen in rabbit cartilage explants cultures. Cartilage explants were left untreated or treated with 5 ng/ml IL-1a for 14 days in the absence or presence of Aralia cordata Thunb.. (A) Proteoglycan was determined by Safranin O staining (Original magnification x200). (B) Collagen expression was determined using Masson's Trichrome staining.

Ⅳ. Discussion

Aralia cordata Thunb. is an oriental medicinal herb used for the treatment of osteoarthritis. However, the current problem facing us is "How can we exert a protective effect on cartilage". Therefore, we investigated the effects of Aralia cordata Thunb. on the release of proteoglycan, the degradation of collagen, and the mechanismsinvolved, in IL-1a-treated rabbit cartilage explants.

In general, the destruction of cartilage in OA is initially caused by a decrease in its proteoglycan content, followed by the degradation of collagen fibers. Some studies have suggested that investigation into cartilage degradation should include an examination of both proteoglycan and the collagen matrix 1, 14). In a preliminary study, we confirmed that proteoglycan is dose-dependently degraded by IL-1a and consistently achieves the maximal response about 73% with 5 ng/ml IL-1a applied to rabbit explants for 14 days of culture (Fig. 1A). Collagen degradation by IL-1asignificantly increased about 72.5% after 14 days (Fig. 1B). Under these conditions, cartilage explants cultured for 28 days still released about 79% of type II collagen into the culture medium (data not shown). Previous investigators have shown that IL-1a induced the degradation of more than 70% of proteoglycan after 3 days and about 65% of collagen after 15-25 days in bovine explants and rabbit cartilage explants[14-15].

We investigated the protective effects of

Baralia cordata Thunb.on IL-1a-mediated proteoglycan and collagen release in rabbit cartilage explants cultures. In this study, Aralia cordata Thunb. dose-dependently reduced IL-1a-mediated proteoglycan release into the culture medium between 3 days and 14 days (Figs. 2 and 3). The release of collagen was not observed in culture medium treated with IL-1a until 7 days. Aralia cordata Thunb. markedly reduced collagen degradation after 14 days in a concentration-dependent manner compared withthat in IL-1a-treated cultures (Figs. 2 and 3). These results suggest that Aralia cordata Thunb. is essential for the reduction of proteoglycan and collagen degradation in rabbit cartilage. The control, diclofenac, a non-selective COX-2 inhibitor, showed inhibitory effects, whereas rofecoxib, a selective COX-2 inhibitor, showed none (Fig. 3). These results are in agreement with those of others[16-18]. Studies by Ito et al. have reported that diclofenac has a positive effect on the inhibition of cartilage metabolism[18].

Aralia cordata Thunb. inhibited MMP-3 and MMP-13 activity in articular cartilage explants (Fig. 4). In our experiments, colorimetric analysis demonstrated that MMP-3 and MMP-13 activities were similarlyinhibited when tested at extract concentrations of 0.02-0.2 mg/ml. These results suggest that Aralia cordata Thunb. is an effective inhibitor of cartilage loss. Proteoglycan is particularly vulnerable to proteinase attack and is therefore a sensitive indicator of proteolytic activity. Studies by Lin et al. have suggested that MMP-3 is the proteinase mainly responsible for the release of proteoglycan and collagen as fragments after cartilage resorption in vitro and in vivo, because they are produced by cleavage of the aggrecan molecule at the position cleaved by MMP-3[19-20]. Furthermore, Kozaci et al. suggested that MMP-13 playsa role in the cartilage destruction stimulated by IL-1b, and breaks down type II collagen breakdown in bovine nasal cartilage explants[21-22].

We have also shown that Aralia cordata Thunb. hadno impact on the viability of cartilage explants, when determined on 3, 7, and 14 daysof the culture period (Fig. 5). The distribution of proteoglycan and collagen determined using Safranin O and Masson's Trichrome staining demonstrated the disorganization of the articular cartilage, including fibrillation, fissures, chondrocyte nuclear cleavage, and cluster formation, in the Aralia cordata Thunb.-treated group compared with the control (Fig. 6). Chondrocytes enlargement, resulting in giant chondrocytes containing multiple nuclei, was also observed in Aralia cordata Thunb.-treated cartilage cultures. Based on the above data regarding the metabolism, viability and morphology of cartilage, we suggest that Aralia cordata Thunb. is potentially useful in the treatment of degenerative joint disease.

However, furtherinvestigation is required into the mechanism of action of Aralia cordata Thunb. in exerting its chondroprotective effect via aggrecanase expression, and to develop an effective regimen for the treatment of osteoarthritis.

V. Conclusion

In summary, Aralia cordata Thunb. has an inhibitory effect on the release of proteoglycan and collagen associated with the downregulation of MMP-3 and MMP-13 activities,without affecting the viability or morphology of IL-1a-induced rabbit articular cartilage explants. We suggest that Aralia

cordata Thunb.could represent agent for pharmacological intervention in cartilage loss in the progress of osteoarthritis.

Ⅵ. Acknowledgements

This study was supported by a grant of the Oriental Medicine R&D project from the Ministry of Health and Welfare, Republic of Korea (03-PJ9-PG6-SO01-0002)

Ⅶ. References

1. Woessner, J.F., Jr, Howell, D.S.. Joint cartilage degradation. New York : Marcel Dekker. 1993 : 1-556.
2. Hardingham, T. E., Fosang, A. J.. The structure of aggrecan and its turnover in cartilage. Journal of Rheumatology Supplement. 1995;43:86-90.
3. Nieduszynski, I. A., Huckerby, T. H., Brown, G. M., Tai, G.-H., Morris, H. G., Eady, S.. There are two major types of skeletal keratan sulphates. Biochemical Journal. 1990;271:243-245.
4. Okada, Y., Shinmei, M., Tanaka, O., Naka, K., Kimura, A., Nakanishi, I., Bayliss, M., Iwata, K., Nagase, H.. Localization of matrix metalloproteinase 3 (stromelysin) in osteoarthritic cartilage and synovium. Laboratory Investigation. 1992;66:1069-1073.
5. Walakovits, L.A., Moore, V.L., Bhardwaj, N., Gallick, G.S., Lark, M.W.. Detection of stromelysin and collagenase in synovial fluid from patients with rheumatoid arthritis and posttraumatic knee injury. Arthritis and Rheumatism. 1992;35:35-42.
6. Ellis, A.J., Curry, V.A., Powell, E.K., Cawston, T.E.. The prevention of collagen breakdown in bovine nasal cartilage by TIMP, TIMP-2 and a low molecular weight synthetic inhibitor. Biochemical Biophysics Research Communication. 1994;201:94-101.
7. Murphy, G, Knuper, V, Atkinson, S, Butler, G, English, W, Hutton, M, Stracke, J., Clark, I.. Matrix metallo proteinase in arthritic disease. 2002;4:s39-49.
8. Saito, S., Katoh, M., Masumoto, M., Matsumoto, S. Masuho, Y.. Involvement of MMP-1 and MMP-3 in collagen degradation induced by IL-1 in rabbit cartilage explant culture. Life Sciences. 1998;62:PL359-365.
9. Green, M.J., Gough, A.K., Devlin, J., Smith, J., Astin, P., Taylor, D., Emery, P.. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. Rheumatology (oxford). 2003;42:83-88.
10. Moldovan, F., Pelletier, J.P., Hambor, J., Cloutier, J.M., Martel-Pelletier, J.. Collagenase-3 (matrix metalloproteinase-13) is preferentially localizedin the deep layer of human arthritic cartilage in situ In vitro mimicking effect by transforming growth factor. Arthritis and Rheumatism. 1997;40:1653-1661.
11. Knauper, V., Lopez-Otin, C., Smith, B., Knight, G., Murphy, G.. Biochemical characterizationof human collagenase-3. Journal of Biological Chemistry. 1996;271:1544-1550.
12. Dang, N.H., Zhang, X., Zheng, M., Son, K.H., Chang, H.W., Kim, H.P., Bae, K., Kang, S.S.. Inhibitory constituents against cyclooxygenases from Aralia cordata Thunb. Arch Pharm Res. 2005;28:28-33.
13. Okuyama, E., Nishimura, S., Yamazaki, M.. Analgesic principles from Aralia cordata Thunb. Chem Pharm Bull. 1991;39:405-407.

14. Kikuchi, T., Sakuta, T., Yamaguchi, T.. Intra-articular injection of collagenase induces experimental osteoarthritis in mature rabbits. Osteoarthritis and Cartilage. 1998;6:177-186
15. Badger, A.M., Cook, M.N., Swift, B.A., Newman-Tarr, T.M., Gowen, M., Lark, M.. Inhibition of interleukin-1-induced proteoglycan degradation and nitric oxide production in bovine articular cartilage/ chondrocyte cultures by the natural product, hymenialdisine. Journal of Pharmacology and Experimental Therapeutics. 1999;290:587-593.
16. Clay, K., Seed, M.P., Clements-Jewery, S.. Studies on interleukin-1b induced glycosaminoglycan release from rat femoral head cartilage in-vitro. Journal of Pharmacy and Pharmacology. 1989;41: 503-504.
17. Yu, L.P., Jr. Smith, G.N., Brandt, K.D., Myers, S.L., O'connor, B.L., Brandt,D.A.. Reduction of the sevierity of canine osteoarthritis by potential chondroprotective agents. Agents Actions. 1993;39:195-206.
18. Ito, A., Nose, T., Takahashi, S., Mori, Y.. Cyclooxygenase inhibitors augument the production of pro-matrix metalloproteinase 9 (progelatinase B) in rabbit articular chondrocytes. FEBS Letters. 1995;360:75-79.
19. Lin, P.M., Christoper Chen, C.T., Torzilli, P.A.. Increased stromyelin-1 (MMP-3), proteoglycan degradation (3B3- and 7D4) and collagen demage in cyclically load injuried articular cartilage. Osteoarthritis and Cartilage. 2004;12:485-496.
20. Dodge, G.R., Jimenez, S.A.. Glucosamin sulfate modulates the levels of aggrecan and matrix metalloproteinase-3 synthesized by cultured human osteoarthritis articular chondrocytes. Osteoarthritis and Cartilage. 2003;11:424-432.
21. Kozaci, L.D.,Brown, C.J., Adcocks, C., Galloway, A., Hollander, A.P., Buttle, D.J.. Stromyelin 1, neutrophil collagenase, and collagenase 3 do not play major roles in a model of chondrocyte mediated cartilage breakdown. Journal of Clinical Pathology. 1998;51:282-286.
22. Shingleton, W.D., Ellis, A.J., Rowan, A.D., Cawston, T.E.. Retinoic acid combines with interleukin-1 to promote the degradation of collagen from bovine nasal cartilage: Matrix Metalloproteinase-1 and 13 are involved in cartilage breakdown. Journal of Cellular Biochemistry. 2000;79:519-531.