2007. Vol. 28. No. 4. *114-123*
Korean Journal of Oriental Medicine

Original Article

Effect of *Aralia Cordata* Pharmacopuncture on Cartilage Protection and Apoptosis Inhibition *In Vitro* and in Collagenased-induced Arthritis Rabbit Model

Yong-Hyeon Baek, Dong-Suk Park

Department of Acupuncture & Moxibustion, College of Oriental Medicine, Kyung Hee University

Osteoarthritis is characterized by cartilage degradation and chondrocytes death. Chondrocyte death is induced by the apotosis through special mechanisms including the activation of caspase-3. On the basis of this background, this study was designed to examine the cartilage protective and anti-apototic effects of *Aralia Cordata* in *in vtro* and in collagenase-induced arthritis rabbit model.

To conduct *in vitro* study, chondrocytes culturedfrom rabbit knee joint were treated by 5 ng/ml IL-1a.For *in vivo* experiment, collagenase-induced arthritis (CIA) rabbit model was made via intraarticular injection with 0.25 ml of collagenase solution. *Aralia cordata* pharmacopuncture (ACP) was administrated on bilateral Dokbi acupoint (ST35) of rabbits at a dosage of 150 ㎍/kg once a day for 28 days after the initiation of the CIA induction.

In the study by using CIA rabbit model *in vivo*, ACP showed the inhibition of cartilage degradation in histological analysis. *Aralia cordata* also showed anti-apoptotic effect both *in vitro* and *in vivo* study. In chondrocytes treated by IL-1a, *Aralia cordata* inhibited caspase-3 activity and enhanced the proliferation of IL-1a-induced dedifferentiated chondrocytes. ACP showed the inhibition effect on the caspase-3 expression and activity from CIA rabbit model.

This study indicates that ACP inhibits the cartilage destruction and the chondrocyte apotosis through downregulation of caspase-3 activity. These data suggest that ACP has a beneficial effect on preventing articular cartilage destruction in osteoarthrtis.

Key Words : *Aralia cordata*, osteoarthritis (OA), cartilage, apotosis, caspase-3, collagenase-induced arthritis (CIA)

Introduction

Osteoarthritis (OA) is characterized by structural and biochemical changes in chondrocytes and cartilages, and insufficient synthesis of extracellular matrix (ECM) because of chondrocyte phenotype loss (i.e. dedifferentiation) and increased numbers of apoptotic chondrocytes[1-5]. Chondrocytes are differentiated from mesenchymal cells during embryonic development. Differentiated chondrocytes synthesize sufficient amounts of cartilage-specific ECM, including type Ⅱ collagen and proteoglycan, to maintain matrix integrity[6,7]. This homeostasis is recognized to be destroyed by chondrocyte apoptosis in the state of OA. The current treatments for OA only modify symptoms rather than underlying processes. Therefore, more suitable therapies that modify the pathophysiology of OA are needed. At this point, chondrocyte apotosis is an essential target in

・received : 15 November 2007
・received in revised from : 17 November 2007
・accepted : 7 December 2007
・Correspondence to : Dong-Suk Park
Department of Acupunture, College of Oriental medicine, KyungHee 149, Sangildong, Kangdonggu, Seoul, South Korea
(Tel : +82-2-440-7702 / Fax : +82-2-440-6799
E-mail : address: dspark49@yahoo.co.kr)

developing treatment of OA.

Chondrocyte apoptosis may be involved in a number of arthritic diseases, such as rheumatoid arthritis[8] and osteoarthritis[9,10]. Recent reports suggest that chondrocyte cell death occurs primarily via apoptosis or apoptosis-like programmed cell death in OA. Also, chondrocyte apoptosis has been positively correlated with the severity of cartilage destruction and with matrix depletion in human specimens of OA[11-14]. Chondrocyte apoptosis regulation is modulated by multiple phosphorylation of several different protein kinases. They are major subtypes of mitogen-activated protein (MAP) kinase, such as extracellular signal-regulated kinase ERK-1 /2, p38 kinase, and caspase-3[15]. It has been also known that the MAP kinase subtypes are activated by proinflammatory cytokines, such as IL-1[16].

Aralia cordata (*A. cordata*) has been used in treatment of arthritis and low back pain. It has been reported that *A. cordata* inhibited COX-2 dependent PGE2 generation[17] and showed effectiveness regarding analgesia, hypothermia, duration of pentobarbital-induced anesthesia[18]. However, there is no report related to the inhibition of chondrocyte apoptosis in OA. Therefore, this is the first study to reveal the inhibition effect of *A. cordata* to chondrocyte apoptosis.

In the present study, we intended to reveal the cartilage-protective and cartilage regenerative effect of *A. cordata* by showing the inhibition of chondrocyte apoptosis. For this, we investigated [1] the inhibition of cartilage degradation in histological analysis (*in vivo*), and[2] the inhibition effect of *A. cordata* pharmacopuncture (ACP) on interleukin-1α-induced chondrocyte apoptosis related with caspase-3 (*in vitro*), and [3] the inhibition effect of ACP on chondrocyte apoptosis in the rabbit model of CIA by using immunohistological and colorimetric analysis (*in vivo*).

Materials and Methods

1. *In Vitro* Study

1) Drugs and reagents

The root of *A. cordata* was extracted at room temperature in 70 % (v/v) ethanol-water for 24 h (productivity of 24.5 %). 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 ℃. 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 0.01 to 100 ㎍/㎖ (in chondrocyte).

2) Isolation and culture of chondrocytes from rabbit articular cartilage

Rabbit articular chondrocytes were cultured from tibial plateaus and femoral condyle in cartilage[19]. Briefly, five-week-old rabbits (Samtako Biokorea Co., Korea) were killed and articular cartilages were removed as mentioned before. Thin slices of cartilage were sequentially digested by 0.2 % collagenase type Ⅱ (Sigma, MI, USA) and the resulting cell suspension was transferred to 75 ㎠ culture flasks, at 105 cells/㎠ (high density) containing 12 ml DMEM medium (Gibco BRL-Life Technologies, USA), 10 % fetal calf serum (FCS), 100 IU/ml penicillin, and 100 mg/ml streptomycin (Roche, Germany) with initial pH 7.2-7.6. Cells were then cultured at 37 ℃ in an atmosphere of 8 % CO2 in air, and the

medium was changed once until confluency (day 6 of the culture).

3) Treatment of chondrocytes with IL-1a, *Aralia cordata*

Chondrocytes (1 × 106/ml) were plated in 6-well plates and serum-starved for 12 h/overnight. The medium was replaced with fresh medium containing recombinant IL-1a(5 ng/ml), and chondrocytes were incubated for 24 h in a tissue culture incubator at 37℃ and 5 % CO2. This concentration of IL-1a was chosen based on pilot experiments performed to determine the lowest concentration of IL-1a that will induce the maximum degradation of proteoglycan and collagen *in vitro*. To study the dose-dependence of the effect of *A. cordata* on IL-1a-induced cartilage destruction, chondrocytes were treated with varying concentrations of *A. cordata* (0.01-100 ㎍/ml).

4) Assay of caspase-3 activity

Caspase-3 activation in IL-1a-treated chondrocytes was determined by measuring the absorbance of a cleaved synthetic substrate of caspase-3, Ac-Asp-Glu-Val-Asp-chromophore p-nitroaniline. Briefly, chondrocytes were lysed on ice for 10 min in cell lysis buffer provided in the Clontech A ApoAlertTM CPP32 colorimetric assay kit. Lysates were reacted with 50 ㎛M Ac-Asp-Glu-Val-Asp-chromophore p-nitroaniline in reaction buffer (0.1 M HEPES, 20 % glycerol, 10 mM dithiothreitol, and protease inhibitors (pH 7.4)). Mixtures were maintained at 37 ℃ for 1 h in a water bath and subsequently analyzed in an enzyme-linked immunosorbent assay reader. Enzyme activity was calculated from a standard curve prepared using p-nitroaniline. The relative levels of p-nitroaniline were normalized against the protein concentration of each extract.

5) Cell proliferation BrdU assay

Chondrocytes were seeded at a density of 5×103 cells/well in 96-well plates in complete M199 medium. After 24 hr, the medium was removed and replaced with M199 medium plus 5 % FCS and stimulators (*A. cordata*, PD98059 and SB203580) in the presence or absence of 5 ng/ml IL-1a in a volume of 100 ㎕. After 48 h incubation at 37 ℃, 10 ㎕ of BrdU were added to each well, and the samples were incubated for a further 6 h at 37 ℃. Cells are fixed and added anti-BrdU-POD and then detected by the TMB substrate reaction. The reaction product is quantified by ELISA reader at 480-650 mm.

2. *In Vivo* Study

1) Experimental animals

Male New Zealand white rabbits were obtained from Samtako Biokorea Co. (Osan, Korea) and housed individually (MJ-161C, Myung Jin Instrument Co., Korea). All animals had free access to tap water and pellet food (Agribrands, Purina, Korea). The room was light/dark (08:00-20:00 h light, 20:00-08:00 h dark) controlled and kept at 21-24 ℃. All experiments were carried out according to the internationally accredited guidelines.

2) Collagenase-induced arthritis(CIA) induction

For the *in vivo* experiment, collagenase was dissolved in saline at a concentration of 4 mg/ml just before useand the solution was filtrated with a 0.22 ㎛ membrane. Rabbits weighing 2.5 3.0 kg were grouped (N=5) and anesthetized with an intramuscular injection of tiletamine-zolazepam (100 ㎎/㎏, Zoletil 50®, Virbac, France). After

shaving and sterilizing, the right knee joint was injected intraarticularly with 0.25 ml of saline or collagenase solution. The injection was perfo-rmed twice on days 1 and 4 according to the method of Kikuchi et al[16,20].

3) ACP preparation and administration

For intradermal injection into rabbits, Ethanol-water extracted *A. cordata* (productivity of 25.4 %) were suspended in 0.5 % carboxymethylcellulose (CMC) at a concentration of 50 ㎍/10 ㎕. ACH were administrated on bilateral Dokbi acupoint (ST35) of rabbits at a dosage of 150 ㎍/kg once a day for 28 days after the initiation of the CIA induction. *A. cordata* extract was suspended in 0.5 % CMC solution and injected intradermally on Dokbi acupoint (ST35). Rabbits in control group were given only 0.5 % CMC.

4) Histopathological examinations

Rabbits were sacrificed at 28 days after collagenase injection. Knee joint was fixed with 10 % formalin were fixed with 10 % neutral buffered formalin (pH 7.4) and decalcified with 20 % EDTA. The decalcified knee joint was embedded in paraffin, and 5 mm microsections of them were prepared and stained with hema-toxylin and eosin (H&E) stain. The weight-bearing regions of cartilage were evaluated by scoring in accordance with the evaluation criteria of Kikuchi, which are a modification of those described by Mankin et al[21]. This system evaluates the severity of lesion based on the loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, disorganization of chondrocytes, loss of chondrocytes, and cluster formation using the modified histologic scoring system of Kikuchi et al[16,20].

5) Immunohistochemical analysis

Rabbit knee joint specimens were fixed in 10 % neutral-buffered formalin for 24 h and were paraffin embedded according to standard procedures. Four micrometer-thick sections of blocks from each case were deparaffinized in xylene, rehydrated, and treated with 3 % hydrogen peroxide (H2O2) in methanol for 10 min to block endogenous peroxidase activity. All sections were subjected to heat-induced epitope retrival in a microwave oven. Sections were incubated with normal goat serum and applied with anti-PCNA and anti-cleaved-caspase-3 antibody for 12 h at 4 ℃. Slides from all case studies were then simultaneously processed using the avidin-biotin complex method peroxidase kit. Negative control was performed using nonimmunized goat serum instead of primary antibody. Anti-PCNA and anti-cleaved-caspase-3-labeled sections were visualized with streptavidin peroxidase and diaminobenzidine, followed by hematoxylin counterstain to render proliferating, or apoptotic nuclei dark brown and normal cell nuclei blue.

6) Assay of caspase-3 activity

Caspase-3 activation in serum of CIA rabbit was determined by measuring the absorbance of a cleaved synthetic substrate of caspase-3, Ac-Asp-Glu-Val-Asp-chromophore p-nitroaniline. Briefly, cells were lysed on ice for 10 min in cell lysis buffer provided in the Clontech A ApoAlertTMCPP32 colorimetric assay kit. Lysates were reacted with 50 ㎛M Ac-Asp-Glu-Val-Asp-chromophore p-nitroaniline in reaction buffer (0.1 M HEPES, 20 % glycerol, 10 mM dithiothreitol, and protease inhibitors (pH 7.4)). Mixtures were maintained at 37 ℃ for 1 h in a waterbath and

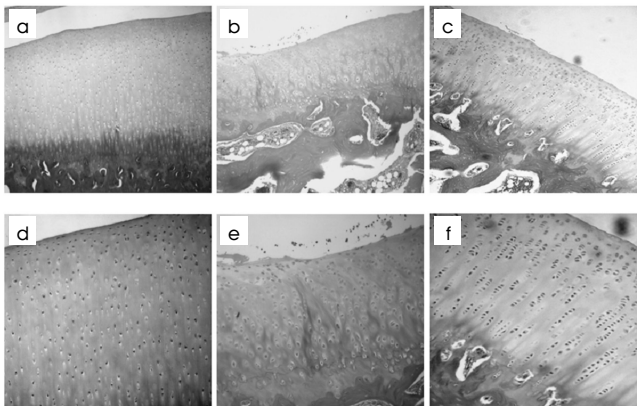

Fig. 1. Histological characteristics of rabbit articular cartilage with various levels of degradation in CIA models (femoral chondyle)
a & d: normal cartilage from vehicle-administrated in rabbit, b & e: cartilage of collagenase-incuced arthritis model, c & f: cartilage of *A. cordata*-administrated rabbit in collagenase-incuced arthritis model
a & b & c : × 10
d & e & f : × 20

subsequently analyzed in an enzyme-linked immunosorbent assay reader. Enzyme activity was calculated from a standard curve prepared using p-nitroaniline. The relative levels of p-nitroaniline were normalized against the protein concentration of each extract.

7) Statistical analysis

All data were represented as means ± SEM. The significance of statistical differences among groups were determined using one way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. And between two groups, the statistically significant differences were determined by student's t-test or paired t-test. Differences were considered significant when p value was less than 0.05.

Results

1. Effects of ACP on cartilage changes in CIA rabbit model

Table 1. Histological Evaluation Scores of Articular Cartilage in Collagenase-induce Arthritis Rabbits (Kikuchi score 1-32, n=5)

	0.5 % CMC	150 μg/kg A.cordata
Femoral condyle		
Loss of superficial layer	3.7 ± 2.1	2.3 ± 1.3*
Erosion of cartilage	3.1 ± 1.7	1.7 ± 0.5**
Fibrillation and /or Fissures	3.3 ± 0.7	1.9 ± 0.8**
Disorganization of chondrocytes	3.9 ± 0.6	2.1 ± 1.1*
Loss of chondrocytes	2.8 ± 0.8	1.1 ± 0.9**
Cluster formation	2.5 ±1.4	1.2 ± 0.4**
Sum score	19.3 ± 7.3	10.3 ± 5.3*

Each datum was represented as mean ± SEM.
*p<0.05, **p<0.01 compared to 0.5 % CMC treated group

Cartilage degradation was assessed according to the Kikuchi score[16], based on the histological assessment through the overall loss of superficial layer, erosion, fissure, disorganization of chondrocytes, and cluster formation. Results of the histopathological evaluation are summarized in Table 1. Cartilages of the dorsal section of femoral chondyle were investigated by H&E stain with light microscope. The cartilage degradation of the tibial plateau and femoral chondyle in the *A. cordata*-treated group significantly reduced (Table 1).

In the 0.5 % CMC-treated group, different degrees of OA-like degenerative changes were investigated in the collagenase-injected rabbit. In the *A. cordata*-treated group, the loss of superficial layer, erosion, fissure, disorganization of chondrocytes, and cluster formations were reduced compared to control group (Fig. 1).

2. Effect of *A. Cordata* on the Proliferation of chondrocytes

The measurement for cell proliferation was performed by BrdU incorporation assay. In both 5 ng/ml IL-1a-treated and IL-1a-untreated chondrocytes, 0.01 100 ㎍/ml *A. cordata* increased cell proliferation in a dose-dependent manner (Fig. 2).

3. Effects of *A. Cordata* on caspase-3 activity in chondrocytes

IL-1a significantly increased the caspase-3 activity in chondrocytes. *A. cordata* with IL-1a dose-dependently decreased the caspase-3 activity (Fig. 3).

4. Effects of ACP on apoptosis in CIA

The anti-apoptotic effect of *A. cordata* was confirmed by CIA model. cleaved caspase-3, the biological relevant biochemical parameters, was

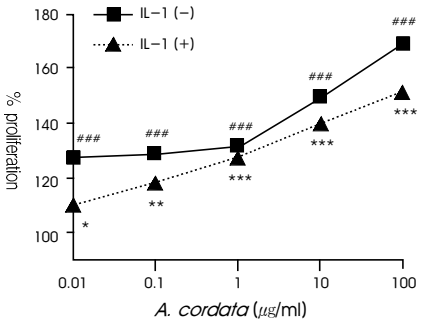

Fig. 2. Effect of *A. cordata* on proliferation of chondrocytes
In both 5 ng/ml IL-1a-treated group and IL-1a-untreated group, Chondrocytes were treated with 0.01-100 ㎍/ml *A. cordata* for 72 h, and then proliferation was assessed by BrdU incorporation assay. Data were obtained from three independent experiments and each datum was represented as mean ± SEM (n=12).
###p<0.001 : compared to control group [without *A. cordata* in IL-1a-untreated group (IL-1(-))]
*p<0.05, **p<0.01 ***p<0.001 : compared to control group [without *A. cordata* in IL-1a-treated group (IL-1 (+))]

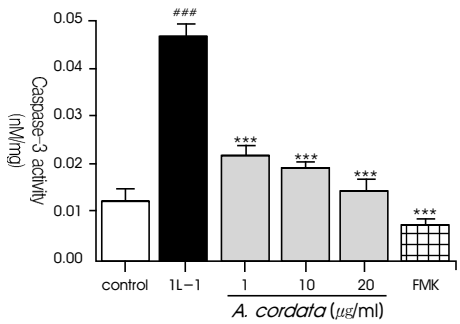

Fig. 3. Effects of *A. Cordata* on caspase-3 activity in chondrocytes
Chondrocytes were treated with various concentrations of *A. cordata* and 100 mM Z-VAD-FMK (caspase-3 inhibitor) for 24h in the prescence of 5 ng/ml IL-1a. Caspase-3 activity was determined using a colorimetric substrate. Each data were represented as mean ± SEM (n=8).
IL-1 : group of IL-1a-treated without *A. Cordata*
###p<0.001 compared with control group
***p<0.001 compared with IL-1

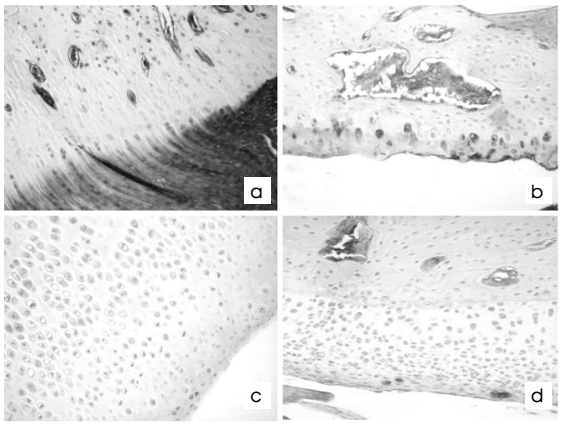

Fig. 4. Immunohistochemical analysis on cleaved caspase-3 expression in CIA models
a & b: CIA cartilage, c & d: administration of *A. cordata* for 4 weaks in collagenase-incuced cartilage, a & c: femoral chondyle b & d: tibial plateau.

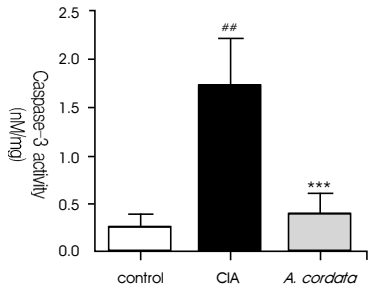

Fig. 5. Effects of ACP on caspase-3 activity in serum from CIA rabbit model
Serums from *A. cordata*-treated and non-treated CIA rabbit model were measured by colorimetric assay. Each data were represented as mean ± SEM (n=5).
Control : normal group without induction of CIA
CIA : CIA group without *A. Cordata* treatment
##p<0.01 compared with control
***p<0.001 compared with CIA

selected as a marker of apoptosis.

In immunohistochemical analysis, cleaved caspase-3 was positively stained in the chondrocytes of superficial layer and cluster forms. In the *A. cordata*-treated group, apoptosis was dramatically reduced in the femoral chondyle and tibial plateau (Fig. 4). Results of the immunohistochemistry evaluation are summarized in Table 2.

In colorimetric analysis, caspase-3 activity was also confirmed from serum of experimental groups.In the CIA group, caspase-3 activity was significantly increased by 6.4 fold compared to control group, and in the *A. cordata*-treated group, caspase-3 activity was significantly inhibited by 4.3 fold compared to CIA group (Fig. 5).

Discussion

Aralia cordata is an oriental medicinal herb which has been used for the treatment of OA. However, the current problem is "How can we prove the cartilage's protective and anti-apoptotic

Table 2. Cleaved Caspase-3 Expression of Rabbit Articular Cartilage in CIA (number of positive cells, n=5)

	0.5 % CMC	150 μg/kg *A.cordata*
Caspase-3		
Femoral chondyle	59.7 ± 4.2	21.7 ± 11.4*
Tibial plateau	83.5 ± 8.8	27.8 ± 5.3**

Each data were represented as mean ± SEM.
*p<0.05, **p<0.01 compared to 0.5 % carboxymethylcellulose(CMC) treated control group

effect of *A. cordata* in OA?" In a preliminary *in vitro* study, we confirmed that *A. cordata* showed the cartilage'sprotective and chondroprotective effect through the inhibition of GAG and collagen degradation, increase of TIMP-1 activity, and decrease of MMPs (MMP- 1,-3,13) activities. And also it was revealed that *A. cordata* inhibited the apoptosis through the downregulation of JNK/p38 MAP kinase signal and the inhibition of caspase-3 activity *in vitro* study using cultured chondrocytes from rabbit articular cartilage. However there was no *in vivo* study related with cartilage protective and anti-apoptotic effect of *A. cordata*.

On basis of the cartilage and chondrocyte protective effect of *A. cordata in vitro*, a further *in vivo* study was conducted to reveal the cartilage protective and anti-apoptotic effect of *A. cordata* in the rabbit model of collagenased-induced arthritis (CIA). CIA has been recognized as a useful OA animal model, since the cartilage degeneration is similar to the corresponding lesion in humans OA[16]. For this, ACPwas administrated on bilateral Dokbi acupoint (ST35) - in general, ST35 is used to treat knee osteoarthritis in Korean Medicine, and located around the knee joint - of rabbits at a dosage of 150 μg/kg once a day for 28 days after the initiation of the CIA induction. Then, cartilage's protective effect was evaluated by histology and immunohistochemistry *in vivo*. In histological assessment, *A. cordata* significantly inhibited the cartilage degradation, such as proteoglycan and collagen, of the femoral condyle. These facts are consistent with the study by Choi et al[22], and also suggest that ACP has the potential effect to ameliorate the progress of OA by protecting the cartilage degradation.

Also, further study was conducted to investigate the inhibitory effect of *A. cordata* on chondrocyte apoptosis in OA. In general, OA is characterized by structural and biochemical changes in chondrocytes and cartilages, including degradation of the cartilage matrix, by increase of apoptotic chondrocytes. Apoptosis is induced by the activation of caspases through the regulation of MAPK signaling pathway, such as ERK-1/2 and p38. Thus, we investigated whether *A. cordata* inhibits the apoptosis by the suppression of caspase-3 activity, and confirmed the inhibition of ACP on the cleaved caspase-3 expression by immnohistochemistry and caspase-3 activity by colorimetric analysis. These results are consistent with *in vitro* data in which *A. cordata* inhibited the activation of caspase-3. Caspase-3 has been known as a final singal of apoptosis. Thus, we propose that *A. cordata* have a direct effect on anti-apoptosis. And *A. cordata* dose-dependently enhanced the proliferation of chondrocytes in treatment with and without IL-1a. This suggests that *A. cordata* can be effective in re-differentiation of chondrocytes. From this result, we realized that *A. cordata* had not only anti-apoptotic effect but also proliferative effect in chondrocytes.

In this study, the anti-apoptotic effect of ACP was confrimed only by the suppression of caspase-3 activation. But, further studies are needed to clarify the other mechanisms related with apoptosis.

In conclusion, ACP inhibits the cartilage destruction and chondrocyte apotosis through downregulation of caspase-3 activity, and enhance the proliferation of chondrocytes. These data suggest that ACP has a beneficial effect on preventing articular cartilage destruction in osteoarthrtis.

Acknowledgements

This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea (B030008)

References

1. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res. 2001; 3(2):107-113
2. Poole AR. An introduction to the pathophysiology of osteoarthritis. Frontiers Biosci. 1999; 4:662-670
3. Frenkel SR, Di Cesare PE. Degradation and repair of articular cartilage. Frontiers Biosci. 1999; 4:671-685
4. Smith RL. Degradative enzymes in osteoarthritis. Frontiers Biosci. 1999; 4:704-712
5. Denko CW, Malemud CJ. Metabolic disturbances and synovial joint responses in osteoarthritis. Frontiers Biosci. 1999; 4:686-693
6. Sandell LJ, Adler P. Developmental patterns of cartilage. Frontiers Biosci. 1999; 4:731-742
7. DeLise AM, Fisher L, Tuan RS. Cellular interactions and signaling in cartilage development. Osteoarthritis Cartilage. 2000; 8(5): 309-334
8. Firestein GS, Yeo M, Zvaifler NJ. Apoptosis in rheumatoid arthritis synovium. J. Clin. Invest. 1995; 96:1631-1638
9. Vignon E, Arlot M, Hartmann DJ, Moyen G, Ville G. Hypertrophic repair of articular cartilage in experimental osteoarthritis. Ann. Rheum. Dis. 1983; 42:82-88
10. Matyas JR, Adams ME, Huang D, Sandell LJ. Discoordinate gene expression of aggrecan and type Ⅱ collagen in experimental osteoarthritis. Arthritis Rheum. 1995;38:420-425
11. Blanco FJ, Guitian R, Vazques-Martul E, de Toro FJ, Galdo F. Osteoarthritic chondrocytes die by apoptosis: a possible pathway for osteoarthritis pathology. Arthritis Rheum. 1998; 41:284-289
12. Kim HA, Lee YJ, Seong SC, Choe KW, Song YW. Apoptotic chondrocyte death in human osteoarthritis. J Rheumatol. 2000; 27:455-462
13. Kim HA, Suh DI, Song YW. Relationship between chondrocyte apoptosis and matrix depletion in human articular cartilage.J Rheumatol. 2001; 28:2038-2045
14. D'Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW. Prevention of chondrocyte apoptosis. J Bone Joint Surg Am. 2001; 83:25-26
15. Fuchs SY, Adler V, Pincus MR, Ronai Z. MEKK1/JNK signaling stabilizes and activates p53. Proc. Natl. Acad. Sci. U. S. A.. 1998; 95:10541-10546
16. Prickett TD, Brautigan DL. Cytokine activation of p38 mitogen-activated protein kinase and apoptosis is opposed by alpha-4 targeting of protein phosphatase 2A for site-specific dephosphorylation of MEK3. Mol Cell Biol. 2007; 27(12):4217-27
17. Dang NH,Zhang X, Zheng M, Son KH, Chang HW, Kim HP, Bae K, Kang SS. Inhibitory constituents against cyclooxygenases form *Aralia cordata* Thunb.. Arch Pharm Res. 2005; 28:28-33
18. Okuyama E, Nishimura S, Yamazaki M. Analgesic principles from *Aralia cordata* Thunb. Chem Pharm Bull. 1991; 39:405-407
19. Corvol MT, Dumontier MF, Rappaport R. Culture of chondrocytes from the proliferative zone of epiphyseal growth plate cartilage from prepubertal rabbits. Biomedicine. 1975; 23:103-107
20. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities

in articular cartilage from osteoarthritic human hips. J Bone Joint Surg. 197153:523-537
21. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. J Bone Joint Surg. 197153:523-537
22. Choi JH, Choi JH, Kim DY, Yoon JH, Youn HY, Yi JB, Rhee HI, Ryu KH, Jung K, Han CK, Kwak WJ, CohYB. Effects of SKI 306X, a new herbal agent, on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model. Osteoarthritis and Cartilage. 2002; 10:471-478