Effect of *Aralia cordata* Extracts on Cartilage Protection and Apoptosis Inhibition

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Cartilage loss in osteoarthritis is characterized by cartilage degradation and chondrocyte death. Cartilage degradation is induced by activation of matrix-metalloproteinases (MMPs) activity and degradation of glycosaminoglycan (GAG) and collagen. Also, chondrocyte death is induced by the apoptosis through the activation of MAP kinase and caspases activities. On the basis of this background, our study was designed to examine the cartilage protective and anti-apoptotic effect of *Aralia cordata*. Cartilage explants and Chondrocytes were cultured from rabbit knee joint cartilage and treated by 5 ng/ml IL-1α. Cartilage and chondroprotective effects of *Aralia cordata* were determined by measuring (1) GAG and collagen expression, (2) GAG and collagen degradation, (3) TIMP and MMPs expression, and (4) TIMP and MMPs activity. Anti-apoptotic effects of *Aralia cordata* were determined by measuring (1) JNK and p38 MAP kinase expression, (2) apoptotic cells by flow cytometry, and (3) caspase-3 activity. In cartilage explants and chondrocytes treated by IL-1α, *Aralia cordata* showed the decrease of GAG and collagen degradation, decrease of MMPs (MMP-1,-3,-13) activity, and increase of TIMP-1 activity in a dose-dependent manner. *Aralia cordata* also showed anti-apoptotic effect by inhibition of early and late apoptotic cells, sub-G1 phase cells, and caspase-3 activity through the downregulation of JNK and p38 MAP kinase signaling pathway. *Aralia cordata* inhibited the cartilage and chondrocyte destruction through the downregulation of MMPs activities and the inhibition of proteoglycan and collagen degradation. Also, *Aralia cordata* inhibited the chondrocyte apoptosis through the downregulation of JNK and p38 MAP kinase signal, and the inhibition of caspase-3 activity.

Key words *Aralia cordata*; interleukin-1α; apoptosis; JNK; p38; caspase-3

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive loss of articular cartilage, chondrocyte destruction, subchondral bone remodeling, spur formation, and synovial inflammation. OA is believed to be a consequence of mechanical and biochemical events that result in an imbalance between the synthesis and degradation of articular cartilage matrix consisting of proteoglycans (PGs), collagens (type II, IX, XI and others) and water.1 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, cartilage and chondrocyte protection is an essential target in developing treatment of OA.

Proteoglycan is a component of the articular cartilage extracellular matrix, providing it with many of its physiochemical properties.2 They exhibit 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 Collagen is another component of the articular cartilage, which primarily consists of type II collagen.4 It plays a role in maintaining integrity of the cartilage matrix and allows proteoglycan to be held in the matrix.5 The matrix metalloproteinases (MMPs) can degrade all components of the extracellular matrix. MMP-1 is upregulated by the proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) related with the breakdown of cartilage collagen in arthritic diseases.6 MMP-3 is capable of cleaving the aggrecan core protein, as well as type II collagen (in the amino-terminal telopeptide). however, its involvement in the degradation of these proteins in cartilage is unclear.7 MMP-13 is the most efficient collagenase against type II collagen and it has an important role in cartilage collagen turnover.8 The activity of MMPs is controlled by a tissue inhibitor of metalloproteinase (TIMP) which inhibits all MMPs at a stoichiometry of 1 : 1 by forming high-affinity complexes. Imbalance between MMPs and TIMPs is well known to be of importance in the progression of OA.9

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.10 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.11 This homeostasis is recognized to be destroyed by chondrocyte apoptosis in the state of OA.

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, JNK, p38 kinase and caspase-3.12,13 Several studies indicate that JNK and p38 kinase activation processes are associated with apoptosis, whereas ERK activation is coupled with cell survival. So coordinated activation and interactions between ERK and p38 MAP kinase allow cells to respond to various genotoxic and survival factors by affecting a number of downstream targets.14,15 It has been also known that the MAP kinase subtypes are activated by proinflammatory cytokines, such as IL-1.

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

In the present study, we intended to reveal the cartilage and chondrocyte protection effects of A. cordata by showing the inhibition of chondrocyte apoptosis. For this, we investigated 1) the cartilage and chondrocyte protection effect of A. cordata in interleukin-1α-induced chondrocytes from rabbit articular cartilage, and 2) the inhibition effect of A. cordata on interleukin-1α-induced chondrocyte apoptosis related with JNK, p38, and caspase-3.

MATERIALS AND METHODS

Preparation of Aralia cordata 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°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 1 to 50 μg/ml.

Cartilage Explants Culture from Rabbit Articular Cartilage 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 penicillin-streptomycin [Gibco BRL, Maryland, U.S.A.]). The samples were then rinsed several times with complete medium and incubated for 12 d at 37°C in a humidified CO₂/95% 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 penicillin-streptomycin).

Treatment of Cartilage Explants with IL-1α and Aralia cordata Approximately 30 mg of cartilage pieces were placed in 48-well plates and treated with various concentrations of extract of A. cordata. After 1 h of pretreatment, 5 ng/ml IL-1α (R&D Systems, Minneapolis, U.S.A.) was added to the culture media, which were then incubated at 37°C for a further 3 d. The supernatants were harvested and replaced with fresh media containing test reagents. These were incubated for a further 25 d, and 3, 7, 14, and 28 d supernatants were collected and stored at −20°C until assayed.

Isolation and Culture of Chondrocytes Rabbit articular chondrocytes were cultured from tibial plateaus and femoral condyle in cartilage. Briefly, five-week-old rabbits (Samtako Biokorea Co., Korea) were killed and articular cartilages were removed. Thin slices of cartilage were sequentially digested by 0.2% collagenase type II (Sigma, MI, U.S.A.) and the resulting cell suspension was transferred to 75 cm² culture flasks, at 10⁵ cells/cm² (high density) containing 12 ml DMEM medium (Gibco BRL-Life Technologies), 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 °C in an atmosphere of 8% CO₂ in air, and the medium was changed once until confluency (day 6 of the culture).

Treatment of Chondrocytes with IL-1α and Aralia cordata Chondrocytes (1×10⁵/ml) were plated in 6-well plates and serum-starved for 12 h/overnight. The medium was replaced with fresh medium containing recombinant IL-1α (5 ng/ml), and chondrocytes were incubated for 24 h in a tissue culture incubator at 37°C and 5% CO₂. This concentration of IL-1α was chosen based on pilot experiments performed to determine the lowest concentration of IL-1α that will induce the maximum degradation of proteoglycan and collagen in vitro. To study the dose-dependent effect of A. cordata on IL-1α-induced cartilage destruction, chondrocytes were treated with varying concentrations of A. cordata (1—50 μg/ml). Controls consisted of chondrocytes incubated in the culture medium without IL-1α or A. cordata.

Effect of Aralia cordata on Cartilage Protection Glysacosaminoglycan (GAG) Degradation Assay: Glysacosaminoglycan levels in the culture medium were determined by the amount of polyanionic material reacting with 1,9-dimethyl-methylene blue, using shark chondroitin sulfate as the standard. Samples were examined spectrophotometrically at 540 nm (Spectramax, Molecular Devices, Sunnyvale, CA, U.S.A.). The percentage recovery was calculated from the peak height of the sample relative to that of the standard.

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.

Colorimetric Analysis of MMP Activity: The levels of MMP activity in the conditioned media were evaluated using an enzyme-linked immunosorbent assay (ELISA) kit (Biomol Research Lab., Inc., PA, U.S.A.) 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-Oc2H5), which is cleaved by stromelysin-1/MMP-3 and collagenase-3/MMP-13. To assess the proteolytic activity, 25 μl of each sample was pipetted into 96 well plates together with each enzyme, buffer and substrate. After 1 h of incubation at 37°C, the amaples 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.

Effect of Aralia cordata on Chondrocyte Protection Measurement of Gene Expression Type II Collagen, GAG, TIMP-1, MMP-1, MMP-3, and MMP-13: RNA was prepared with Trizol® reagent (Invitrogen Corporation, CA, U.S.A.). Reverse transcription of 1 μg of total RNA was carried out for 60 min at 42°C, and then 15 min at 72°C, using the system for RT-PCR (TaKaRa Biotechnology, Seoul, Korea). PCR using specific primers for each cDNA was carried out in a PCR reaction volume of 10 μl (as supplied by TaKaRa, Korea). Amplification reactions were performed by using type II Collagen, GAG, TIMP-1, MMP-1, MMP-3 and
MMP-13 primers (R&D Systems Inc., MN, U.S.A.). An equal volume from each PCR was analyzed by 1.5% agarose gel electrophoresis, and ethidium bromide-stained PCR products were evaluated. Marker gene expression was normalized to GAPDH expression in each sample. Signal intensity was quantified with the Gel Doc EQ (BIO-RAD Laboratories, Milan, Italy).

Measurement of Glycosaminoglycan (GAG) and Collagen Degrading Activities: GAG levels in the culture medium were determined by the amount of polyanionic material reacting with 1,9-dimethylmethlene blue, using shark chondroitin sulfate as the standard. Samples were examined spectrophotometrically at 540 nm (Spectramax, Molecular Devices, Sunnyvale, CA, U.S.A.). The percentage recovery was calculated from the peak height of the sample relative to that of the standard.

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Measurement of MMP Activity: The levels of MMP activity in the conditional media were evaluated using an enzyme-linked immunosorbent assay (ELISA) kit (Biomol Research Lab., Inc., PA, U.S.A.) according to the manufacturer’s instructions. Briefly, MMP activity was measured using a thiopeptolide as a colorimetric substrate (Ac-Pro-Leu-Gly-[2-mercaptop-4-methyl-pentanoyl]-Leu-Gly-OC2H5), which is cleaved by stromelysin-1/MMP-3 and collagenase-3/MMP-13. To assess the proteolytic activity, 25 μl of each sample was pipetted into 96 well plates together with each enzyme, buffer and substrate. After 1 h of incubation at 37 °C, the samples were measured at 405 nm. For each sample, MMP-1, MMP-3, and MMP-13 activities were measured as a percentage of the MMPs in that culture well.

Effect of Aralia cordata on Apoptosis Inhibition Western Blotting: Cells were prepared by extracting proteins using a lyses buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM EDTA, supplemented with protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin A, and 1 mM of 4-(2-aminooethyl) benzenesulfonyl fluoride) and phosphatase inhibitors (1 mM NaF and 1 mM Na3VO4). After centrifugation, the pellet was resuspended in the loading buffer containing 50 mM Tris–HCl (pH 6.8), 4% glycerol, 2 mM EDTA, 3% SDS, and 0.01% bromphenol blue. The proteins were size-fractionated by SDS-polyacrylamide gel electrophoresis and transferred to Hybond-C nitrocellulose membranes for 2 h at 300 mA using a transfer system. Membranes were blocked with 5% nonfat dry milk in TBS-T (0.05% Tween-20, 138 mM NaCl, and 25 mM Tris base). Protein expression was determined using antibodies purchased from the following sources: rabbit anti-phospho ERK-1/2, JNK and p38. The blots were incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG and enhanced with chemiluminescence (ECL) detection system (Amersham Life Science, Alington Heights, IL U.S.A.).

Measurement of Apoptotic Cells by Flow Cytometry: For flow cytometric analysis of sub G1 cell counting with fragmented DNA, 5×10⁵ cells/well onto 6-well plates were collected at different concentrations for 24 h. The cells were harvested and incubated with 1 ml of 75% cold ethanol for 2 h at −20 °C and then washed with PBS. Cell pellets were incubated with 10 μg/ml RNase before adding 50 μg/ml propidium iodide (PI). Samples were analyzed on a FACSort flow cytometer using the cell quest analysis program (Becton Dickinson, San Jose, CA, U.S.A.), which is also used to determine the percentage of sub GI cells. PI was excited at 488 nm, and fluorescence was analyzed at 620 nm.

Annexin V Staining: To quantify the percentage of cells undergoing apoptosis, we also used Annexin V-FITC kit (Caltag, U.S.A.) as manufacture described. Briefly, chondrocyte cells were incubated for 24 h with IL-1 α, A. cordata, kinase inhibitors (PD98059, SP600125, SB203580). Then the cells were washed twice with cold PBS and resuspended in blinding buffer at a concentration of 5×10⁵ cells/ml. After incubation, 100 μl of the solution was transferred to a 5 ml culture tube, and 5 μl of Annexin V-FITC and 10 μl of PI were added. The tube was gently vortexed and incubated for 15 min at room temperature in the dark. At the end of incubation, 400 μl of binding buffer was added, and the cells were analyzed immediately by flow cytemetry.

Measurement of Caspase-3 Activity: Caspase-3 activation in IL-1α-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 ApoAlert™ CPP32 colorimetric assay kit. Lysates were reacted with 50 μμ Ac-Asp-Glu-Val-Asp-chromophore p-nitroaniline in reaction buffer (0.1 mM HEPES, 20% glycerol, 10 mM diithiothreitol, and protease inhibitors (pH 7.4)). Mixtures were maintained at 37 °C 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.

Statistical Analysis: All data were represented as means± S.E.M. 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

Effect of Aralia cordata on Glycosaminoglycan (GAG) and Collagen Degradation in Cartilage Explants Cultures

To study whether A. cordata affects GAG and collagen degradation in rabbit cartilage explants, rabbit cartilage explants were cultured in the presence of 5 ng/ml IL-1α for 28 d. A. cordata reduced the IL-1α-mediated GAG release into the culture medium until 14 d. No release of collagen into the culture medium was observed from explants treated with 5 ng/ml IL-1α up to 7 d. After 14 d, A. cordata markedly reduced collagen degradation relative to that in the IL-1α-treated cultures, and significantly reduced up to 28 d (data are not shown). A. cordata dose-dependently reduced IL-1α-me-
diated GAG and collagen release into the culture medium. *A. cordata* 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 d. (Fig. 1).

**Effect of *Aralia cordata* on MMP Activity in Cartilage Explants Cultures**

We examined whether *A. cordata* inhibited IL-1α-mediated TIMP-1, MMP-1, MMP-3 and MMP-13 activities in the culture medium. We tested the levels of TIMP-1, MMP-1, MMP-3 and MMP-13 activities in the IL-1α-treated culture media after 14 d with or without *A. cordata* treatment. In the *A. cordata*-treated culture media, MMP-1, MMP-3 and MMP-13 levels were significantly decreased in a dose dependent manner compared with the group of IL-1α-treated culture media without *A. cordata* treatment. And TIMP-1 level was significantly increased dose-dependently compared with the group of IL-1α-treated culture media without *A. cordata* treatment (Fig. 2).

**De-differentiation Induced by IL-1α and Serial Passage of Chondrocytes**

We examined the de-differentiation followed by IL-1α & serial passage in primary culture of articular cartilage. In this experiment, we knew that 5 ng/ml IL-1α induce the definite de-differentiation of chondrocytes in both passage #1 and passage #5, and that the degree of de-differentiation was more severe in passage #5 (data are not shown). On the basis of this result, we used the sample induced by 5 ng/ml IL-1α in passage #5.

**Effects of *Aralia cordata* on the Gene Expression and Activity of GAG, Collagen, TIMP, and MMPs**

The effects of *A. cordata* on the gene expressions of GAG, collagen, TIMP, and MMPs were investigated with increasing dosages. The expressions of collagen and proteoglycan were increased by *A. cordata* treatment in a dose-dependent manner (Fig. 3). *A. cordata* increased the gene expression of TIMP-1 and reduced the expressions of MMP-1, MMP-3, and MMP-13 in a dose-dependent manner (Fig. 4).

**Effects of *Aralia cordata* and MAP Kinase Inhibitors on Chondrocyte Apoptosis**

Chondrocytes were exposed to *A. cordata* and MAP kinase inhibitors, such as PD98059, SP600125 and SB203580, in order to identify the roles of ERK-1/2, JNK, and p38 signaling pathways during IL-1α-induced apoptosis. When chondrocytes were treated with 5 ng/ml IL-1α, ERK-1/2 activity was decreased, as determined by phosphorylation status of the protein. However, *A. cordata* increased the ERK-1/2 activity compared to the IL-1α-treated group. On the contrary, 5 ng/ml IL-1α treatment
increased phospho JNK and p38 activity. In this case, *A. cordata* decreased the phospho JNK and p38 activity compared to the IL-1α-treated group (Fig. 5).

**Effects of Aralia cordata on Chondrocyte Apoptosis in Flow Cytometric Analysis** IL-1α increased apoptotic sub-G1 phase cells in a dose-dependent manner. However, there were no significant differences in a dose over 5 ng/ml (data are not shown). *A. cordata* in the presence of 5 ng/ml IL-1α markedly decreased apoptotic sub-G1 phase cells by propidium iodide (PI) staining using flow cytometric analysis. SP600125, and SB203580, JNK and p38 MAP kinase inhibitor, also decreased apoptotic sub-G1 phase cells. However, PD98059, ERK MAP kinase inhibitor, markedly increased apoptotic sub-G1 phase cells (Fig. 6A). Inhibitory effect of *A. cordata* on apoptosis also confirmed using Annexin V staining to detect externalization of phosphatidylserine (PS) on the cell membrane. *A. cordata* in the presence of 5 ng/ml IL-1α markedly decreased apoptotic cells. Also, SP600125 and SB203580 decreased apoptotic cells, but PD98059 increased apoptotic cells (Fig. 6B).

**Effects of Aralia cordata on Caspase-3 Activity in Chondrocytes** IL-1α significantly increased the caspase-3 activity in chondrocytes. *A. cordata* in the presence of 5 ng/ml IL-1α dose-dependently decreased the caspase-3 activity. SP600125 and SB203580, JNK and p38 MAP kinase inhibitor, also dose-dependently decreased the caspase-3 activity. In contrast, PD98059, ERK MAP kinase inhibitor, significantly increased the caspase-3 activity in a dose-dependent manner (Fig. 7).

**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 protective effect of *A. cordata* in OA?” Moreover, there have been no studies related to the chondroprotective and anti-apoptotic effect of *A. cordata*. Therefore, we investigated the cartilage protective and anti-apoptotic effects of *A. cordata* in *in vitro* study by using cartilage explants cultures and chondrocytes from rabbit articular cartilage.

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 examination of both proteoglycan and collagen matrix should be included to reveal the cartilage destruction.

In a preliminary study, we confirmed that glycosaminoglycan (GAG), a component of proteoglycan, and collagen were dose-dependently degraded by IL-1α. Also, maximal degradation was induced at the concentration of 5 ng/ml IL-1α, 14 d after the administration to rabbit cartilage explants (data are not shown). Also, we confirmed that *A. cordata* had no toxicity in concentration range from 1 to 100 μg/ml by MTT assay (data not shown). On basis of such result, we investigated the inhibitory effects of *A. cordata* on IL-1α-induced GAG and collagen release in rabbit cartilage explants cultures. In this study, *A. cordata* dose-dependently reduced IL-1α-induced GAG and collagen release into the culture medium on 14 d (Figs. 1, 2). These results suggest that *A. cordata* can be effective for reduction of GAG and collagen degradation in rabbit cartilage.
selective COX-2 inhibitor as a control, showed inhibitory effect and this result is consistent with the report in which diclofenac showed inhibitory effect on cartilage metabolism.\textsuperscript{20)}

\textit{A. cordata} also dose-dependently increased TIMP-1 activity, and inhibited MMP-1, MMP-3 and MMP-13 activities in articular cartilage explants (Fig. 2). These results suggest that \textit{A. cordata} showed the inhibitory effect on cartilage loss. Proteoglycan is particularly vulnerable to protease attack and is, therefore, a sensitive indicator of proteolytic activity. Studies by Lin \textit{et al.} suggested that MMPs, as proteinases, were responsible for the release of proteoglycan and collagen.\textsuperscript{1,22)} Furthermore, Kozaci \textit{et al.} suggested that MMPs were related to the cartilage destruction stimulated by IL-1\(\alpha\), type II collagen breakdown in bovine nasal cartilage explants.\textsuperscript{23,24)}

In preliminary study, on 5 d of chondrocyte passage #0, round-shape normal chondrocytes were observed. IL-1\(\alpha\) and serial passages induced de-differentiation of chondrocytes in primary culture of rabbit articular chondrocytes. On basis of such result, we used the choncrocytes of passage #5 with IL-1\(\alpha\) treatment to reveal the change of gene expression of GAG, collagen, TIMP-1, and MMPs by IL-1\(\alpha\) treatment. \textit{A. cordata} treatment markedly increased the expressions of GAG, collagen, and TIMP-1 which were reduced by IL-1\(\alpha\) treatment. Also, \textit{A. cordata} inhibited the expressions and activities of MMP-1, -3, and -13 which were increased by IL-1\(\alpha\) (Figs. 3, 4). These findings suggest that \textit{A. cordata} showed the chodroprotective effect through the regulation of GAG, collagen, TIMP-1, and MMPs activity.

On the basis of the chondroprotective effect of \textit{A. cordata}, we intended to investigate the inhibitory effect of \textit{A. cordata}
on chondrocyte apoptosis. In general, OA is characterized by structural and biochemical changes in chondrocytes and cartilages, including degradation of the cartilage matrix, because of increased numbers of apoptotic chondrocytes. Apoptosis is induced by the activation of caspases through the regulation of MAPK signaling pathway, such as ERK-1/2, JNK, and p38. Thus, we investigated whether *A. cordata* inhibits the apoptosis by the suppression of caspase-3 activity.

*A. cordata* increased the phosphorylation of ERK-1/2 and decreased the phosphorylation of JNK and p38 kinase (Fig. 5). ERK-1/2 activates anti-apoptotic signal, whereas JNK and p38 kinase acts as an induction signal for apoptosis. This fact implies that *A. cordata* showed the anti-apoptotic effect by suppression of JNK and p38 expression. Also, in flow cytometric analysis, we confirmed that *A. cordata* markedly decreased IL-1α induced apoptotic cells (Fig. 6). IL-1α treatment induced apoptosis via caspase-3 activation, and *A. cordata* inhibited the caspase-3 activity in a dose-dependent manner (Fig. 7). PD98059, ERK-1/2 inhibitor, increased the activation of apoptotic sub-G1 cells and caspase-3 activation. On the contrary, SP600125, JNK inhibitor, and SB203580, p38 inhibitor, decreased the activation of apoptotic sub-G1 cells and caspase-3 activity. These data are consistent with the study in which the inhibition of ERK with PD98059 activated the pro-apoptotic signal, and the inhibition of JNK and p38 phosphorylation induced the anti-apoptotic signal. On the basis of these results, we knew that *A. cordata* showed the anti-apoptotic effect through JNK and p38 MAPK pathway.

In conclusion, *A. cordata* showed the cartilage 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, *A. cordata* inhibited the apoptosis through the downregulation of JNK/p38 MAP kinase signal and the inhibition of caspase-3 activity in *in vitro* study using cultured chondrocytes from rabbit articular cartilage.

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