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Chondroprotective Role of Sesamol by Inhibiting MMPs Expression via Retaining NF-κB Signaling in Activated SW1353 Cells

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ABSTRACT: Overexpression of matrix metalloproteinases (MMPs) is a major pathological factor causing cartilage destruction in osteoarthritis (OA). This study aimed to investigate the effects and mechanisms of sesamol on expression of MMPs in activated chondrosarcoma cells. Sesamol significantly attenuated TNF-α- and IL-1β-induced gelatinolysis and expression of MMP-9 in a concentration-dependent manner in SW1353 cells. Additionally, both MMP-1 and -13 stimulated by PMA were inhibited by sesamol. On the other hand, the NF-κB signaling activation through IκB-α degradation was restored by sesamol under TNF-α or PMA stimulation. Furthermore, this bioactive compound exerted the reduction on phosphorylation of ERK1/2 or p38 MAPKs after either PMA or IL-1β stimulation. This study also evaluated whether sesamol down-regulates MMP expression in the joint cartilage of monosodium iodoacetate (MIA)-induced OA in rats. Sesamol prevented the expression of MMP-1 and -9 in the cartilage of MIA-induced OA in rats. The results of this study demonstrate that sesamol inhibits cytokine- or PMA-induced MMPs expression through the signal pathways of either NF-κB or ERK/p38 MAPKs down-regulation. This study also showed that sesamol attenuates destructive factor expression in vivo, providing a potential strategy for the chondroprotective therapy in OA.

KEYWORDS: human chondrosarcoma cells, sesamol, TNF-α, IL-1β, PMA, MMPs, MAPKs, IκB-α

INTRODUCTION

Osteoarthritis (OA) is the most common form of joint disease and is widespread in the elderly population;[1] it affects the weight-bearing joints (knees, hips) and is associated with degeneration of the articular cartilage and subchondral bone. Cartilage resorption is also a hallmark of osteoarthritis, although the pathogenic processes are quite different. A number of mechanisms are thought to be concerned in the destruction of cartilage, which can be categorized as intrinsic or extrinsic. Intrinsic resorption takes place when chondrocytes, the only cells present in the articular cartilage, exert their capacity to affect extracellular matrix resorption. Under the influence of cytokines, in particular, tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6, these cartilage cells switch into a catabolic mode and degrade the surrounding extracellular matrix.[2] In contrast, extrinsic resorption is mediated by tissues or cells that lie outside the articular cartilage.

The matrix metalloproteinases (MMPs) are a family of enzymes that facilitate cartilage turnover and breakdown; their levels are elevated in joint tissues of patients with rheumatoid arthritis (RA) and OA.[3,4] These proteolytic enzymes attack and degrade components of the extracellular matrix. Importantly, they contribute to collagen type II and other matrix protein breakdown. Inflammatory cytokines, such as IL-1β and TNF-α, have been reported to stimulate inducible expression of MMPs (1, 3, 9, and 13) in cartilages.[5] Among the MMPs, collagenases are predominantly important because of their ability to cleave fibrillar collagen, which is the most abundant component of the extracellular matrix.[6] MMP-1 (collagenase-1) is expressed ubiquitously and is found in various cells, including chondrocytes.[7] MMP-13 (collagenase-3) has long been regarded as the major source of collagen degrading activity, because it has preferential capacity to degrade type II collagen.[8] Phorbol 12-myristate 13-acetate (PMA), as a protein kinase C (PKC) activator, was reported to be involved in the expression of MMPs in activated human chondrocytes.[9]

In response to IL-1β and TNF-α, a human chondrosarcoma cell line, SW1353, has been demonstrated to serve as a model that is compatible with primary chondrocytes in OA.[9] It is also well documented that IL-1 and TNF-α are capable of activating the mitogen-activated protein (MAP) kinase family including extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinases, and c-Jun N-terminal kinase (JNK) in human chondrocytes.[10] IκBα is reported to play a significant role in patients with arthritis due to its inhibition of MMP-1 and MMP-13 production.[11] Numerous inhibitors of the MMPs have been proposed as potential therapeutic agents, and the various types of compounds and their activities have been reviewed.[12]

Administration of chondroprotective substances can be used to counteract and/or block the actions of pro-inflammatory cytokine. Sesame (*Sesamum indicum* Linn., Pedaliaceae) has been categorized as a traditional health food in India and other East Asian countries.[13] Sesamol is the major constituent of sesame

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seed oil; it is more resistant to oxidative deterioration than other vegetable oils.[14] Sesamol has been shown to possess neuroprotective,[15] hepatoprotective,[16] anti-inflammatory,[17] chemopreventive,[18] and antiaging properties.[19] A study has also reported that sesamin inhibits lipopolysaccharide-induced cytokine production by suppression of p38 mitogen-activated protein kinase and nuclear factor-κB.[20] In our recent study, we found that sesamol strongly attenuated the signaling of platelet and its aggregation.[21] A recent study has also demonstrated the up-regulation of MMPs in knee cartilage from monosodium iodoacetate (MIA)-injected rats and inhibition of the degenerative changes by several MMP inhibitors.[22] Hitherto, research has tended to focus on cytokine-induced expression of MMPs and their suppression by some natural compounds on chondrocytes in protecting of cartilages; however, there is relatively no information about the inhibitory mechanisms of sesamol on inducible MMPs in chondrosarcoma cell lines, SW1353, in terms of protecting cartilage lesion. Hence, the present study was undertaken to investigate the effects of sesamol on MMPs in chondrosarcoma cell lines, SW1353, under IL-1β, TNF-α, and PMA stimulation and on MIA-induced OA in the rat animal model.

MATERIALS AND METHODS

Materials. Sesamol, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium dodecyl sulfate (SDS), phenylmethanesulfonyl fluoride (PMSF), leupeptin, aprotinin, sodium fluoride, sodium orthovanadate, sodium pyrophosphate, diethyl pyrocarbonate (DEPC), phorbol 12-myristate 13-acetate (PMA), and bovine serum albumin (BSA) were all purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human TNF-α was from Pepro Tech EC (London, U.K.). Anti-mouse and anti-rabbit immunoglobulin G (IgG)-conjugated horseradish peroxidase (HRP) was purchased from Amersham Biosciences (Sunnyvale, CA) and/or Jackson-ImmunoResearch (West Grove, PA). A mouse monoclonal antibody (mAb) specific for human native 92 kDa MMP-9 was purchased from LabVision/NeoMarkers (Fremont, CA). A rabbit polyclonal antibody (pAb) specific for IκBα was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-p38 MAPK and anti-phospho-c-Jun N-terminal kinase (JNK) (Thr183/Tyr185) mAbs, and the anti-phosphop44/p42 extracellular signal-regulated kinase (ERK) (Thr202/Tyr204) polyclonal antibody were from Cell Signaling (Beverly, MA); the Hybond-P PVDF membrane, ECL Western blotting detection reagent, and analysis system were from Amersham (Buckinghamshire, U.K.). All other chemicals used in this study were of reagent grade. Sesamol was dissolved in 0.5% dimethyl sulfoxide (DMSO) and stored at 4 °C until used in in vitro studies.

Cell Cultivation. Human chondrosarcoma cells, SW1353, were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in Ham's F-12 and DMEM (1:1) supplemented with L-glutamine (3.65 mM), penicillin (90 units/ml), streptomycin (90 μg/mL), HEPES (18 mM), $NaHCO_3$ (23.57 mM), and 10% heat-inactivated fetal bovine serum (FBS) at 37 °C in humidified air with 5% CO_2.

Stimulation Experiments. For stimulation of inflammatory agent and cytokines, chondrosarcoma SW1353 cells were seeded at 2.5×10^6 per well of Costar 6- well tissue culture plates in complete media until a confluence of 85% was reached (usually for 24 h). After 24 h, cells were changed to serum-free media. Cells were treated with sesamol (5−20 μM) for 15 min after 24 h of changing serum-free media and then treated with PMA (10 ng/mL), TNF-α (30 ng/mL), and IL-1β (30 ng/mL) for another 24 h. At the end of the incubation period, conditioned medium and cell supernatants were collected and stored at −80 °C for gelatin zymography and Western blotting assay, respectively.

Viability Assay. The cytotoxic effects of sesamol against the SW1353 cell line were determined by the MTT method as described previously.[23] Briefly, cells (2×10^6 cells/mL) were incubated in 12-well plates with different concentrations (5−20 μM) of sesamol for 24 h at 37 °C. At 22 h, the MTT solution was added to each well as a final concentration of 0.5 mg/mL. After 2 h incubation at 37 °C, the supernatant was discarded and replaced with DMSO to dissolve the formazan product, which was measured at 550 nm in a spectrophotometric plate reader. The following formula was used to calculate the percentage of cell viability: percentage cell viability = (absorbance of the experiment samples/absorbance of the control) × 100%.

Gelatin Zymography. MMP-9 expression was detected by gelatin zymography as described by Chung et al.[24] The conditioned medium was mixed with nonreducing buffer (500 mM Tris-HCl, 25% glycerol, 10% SDS, and 0.32% bromophenol blue; pH 6.8) and electrophoresed in 10% polyacrylamide gel containing gelatin (1 mg/mL). After electrophoresis, the gels were washed two times with 2.5% Triton X-100 to remove the SDS and then incubated with reacting buffer containing 50 mM Tris-base, 200 mM NaCl, 5 mM $CaCl_2$, and 0.02% Brij 35 (pH 7.5) for 17 h in a closed container at 37 °C. At the end of the incubation, the gels were fixed with a fixing solution (7% acetic acid and 40% methanol, v/v) for 30 min. Gels were stained with a solution of Colloidal Brilliant Blue G in 27% methanol for 30 min or longer. Finally, a destaining solution (10% acetic acid in 25% methanol) was used to adjust the clear conditions. Clear zones (bands) against the blue background indicated the presence of degradative activity of MMP-9.

SDS−Polyacrylamide Gel Electrophoresis (PAGE) and Western Blot Analysis. Western blot analyses were performed as previously described.[23] Lysates from each sample were mixed with 6× sample buffer (0.35 M Tris, 10% w/v SDS, 30% v/v glycerol, 0.6 M DTT, and 0.012% w/v bromophenol blue, pH 6.8) and heated to 95 °C for 5 min. Proteins were separated by electrophoresis and transferred onto nitrocellulose membranes (for MMP-9) and polyvinylidene difluoride (PVDF) membranes (for MMP-1/-13, p38, pERK1/2, c-JUN, and IκB-α). The membranes were blocked with 5% nonfat milk in TBS−0.1% Tween 20 and sequentially incubated with primary antibodies and HRP-conjugated secondary antibodies, followed by enhanced chemiluminescence (ECL) detection (Amersham Biosciences). BIO-PROFIL Bio-1D light analytical software (Vilber Lourmat, Marue La Vallee, France) was used for the quantitative densitometric analysis. Data of specific protein levels are presented as relative multiples in relation to the control.

Induction of Iodoacetate-Induced Osteoarthritis and Treatment in Vivo. Male Wistar rats (300−450 g) were used for the experiments. All animal experiments and care were performed according to the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996). All procedures were approved by the Animal Care and Use Committee, LAC-98-0036. The animals were acclimated for 1 week prior to dosing, during which time they had free access to food and water ad libitum. Eighteen such acclimated rats were divided into three groups of six each: group I, normal rats (received saline); group II, monosodium iodoacetate (MIA)-induced untreated rats; group III, MIA-induced rats treated with sesamol.

Arthritis was induced by a single intraarticular injection of iodoacetate into the knee joint of anesthetized rats.[22] A 10 mg/mL concentration of MIA (Aldrich Chemical, Milwaukee, WI) was prepared using injectable saline as the vehicle. After appropriate anesthesia (1 mL/kg bw, of 40% chloral hydrate), each rat was positioned on their back and the left leg was flexed 90° at the knee. The patellar ligament was palpated below the patella, and the injection was made into this region. Each rat received a 25 μL intraarticular injection of MIA into the left knee using a glass gastight syringe with a 0.5 in. needle for 7 days and then was treated with sesamol dissolved in saline (30 mg/kg bw) by oral feeding twice a week for 2 weeks. After 2 weeks of sesamol

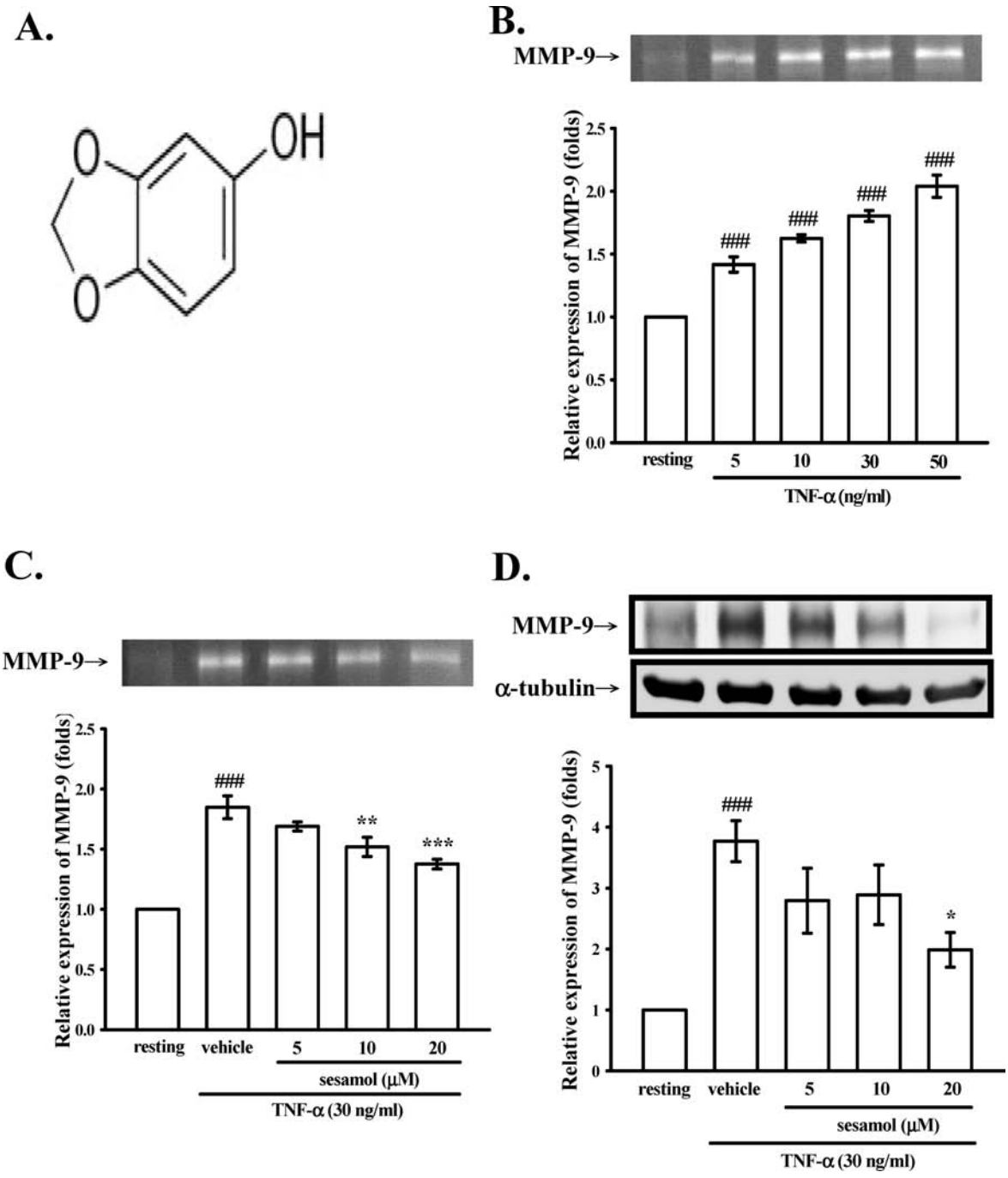

Figure 1. Effects of sesamol on tumor necrosis factor (TNF)-α-induced expression of MMP-9 in SW1353 chondrosarcoma cell line: (A) structure of sesamol; (B) cells were treated with various concentrations of TNF-α for 24 h (cell-free conditioned media were assayed for MMP-9 activation by gelatin zymography); (C) effects of sesamol on TNF-α-induced activation of MMP-9 (cells were pretreated with DMSO or various concentrations of sesamol for 15 min before treatment with TNF-α (30 ng/mL) for 24 h); (D) cell lysates were obtained and analyzed for MMP-9 protein expression by Western blotting (bottom, densitometric analysis of bands for MMP-9, relative to α-tubulin normalized to the resting condition). ###, $P < 0.001$ compared with the resting group; ∗, $P < 0.05$, ∗∗, $P < 0.01$, and ∗∗∗, $P < 0.001$, compared with the vehicle group.

treatment, animals were sacrificed, and the left joint was immediately disarticulated prior to the following analyses.

Preparation of Cartilage Extracts. The tissue surrounding the joint was removed, the joint was disarticulated, and the cartilage was scraped from the surface of the tibia and femur using a scalpel blade and an illuminated magnifier. The cartilage chips were placed into a preweighed vial, the weight of the cartilage was determined, and cold extractant was added to the vial. MMPs were extracted using a two-step procedure. Ten microliters of 0.25% Triton X-100 with 0.01 M $CaCl_2$ was added per milligram of cartilage. Extraction was performed for 20 min at 4 °C followed by centrifugation in a microfuge for 20 min at 13000 rpm. The supernatant was removed and the pellet extracted with 50 mM Tris-HCl, 0.1 M $CaCl_2$, 0.15 M NaCl, pH 7.5. The extract was heated for 4 min at 60 °C in a water bath and centrifugation repeated as described above. The supernatant was collected and combined with the supernatant from the Triton-X 100 extract and was utilized for Western blot analysis for MMPs as described above.

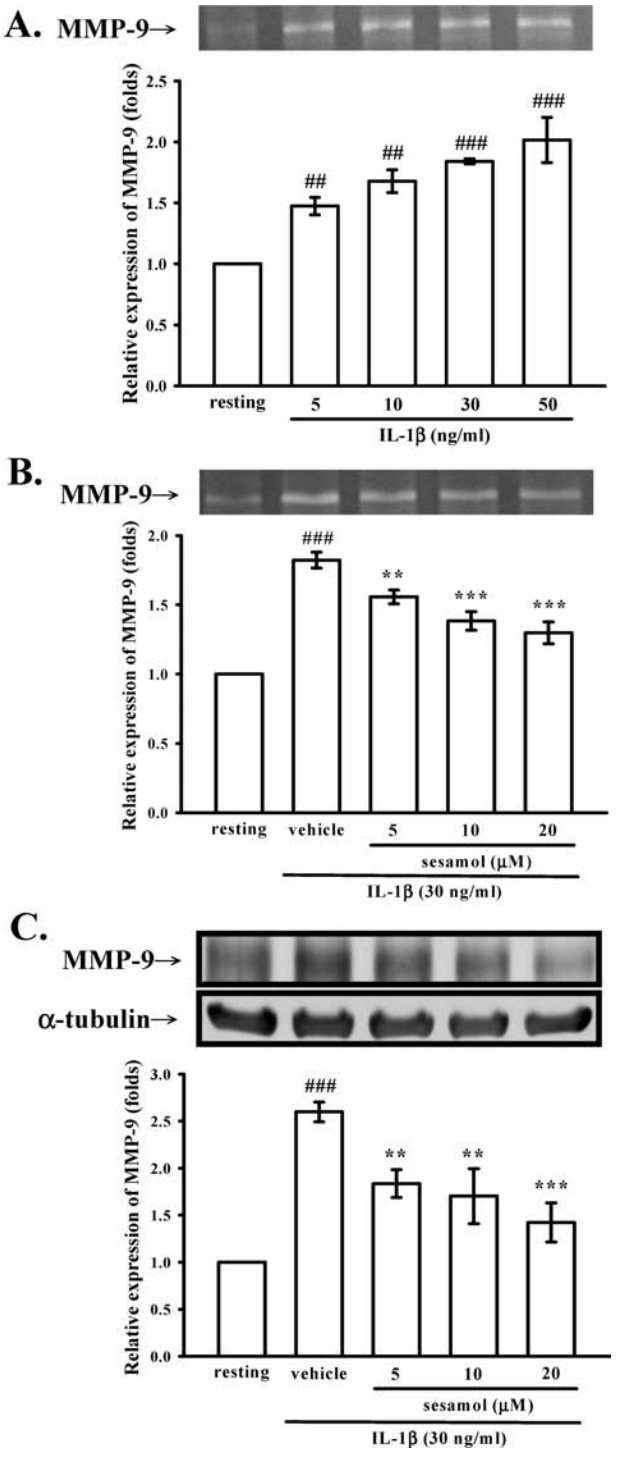

Figure 2. Effects of sesamol on IL-1β-induced expression of MMP-9 in SW1353 chondrosarcoma cell line: (A) cells were treated with various concentrations of IL-1β for 24 h (cell-free conditioned media were assayed for MMP-9 activation by gelatin zymography); (B) effects of sesamol on IL-1β-induced activation of MMP-9 (cells were pretreated with DMSO or various concentrations of sesamol for 15 min before treatment with IL-1β (30 ng/mL) for 24 h); (C) cell lysates were obtained and analyzed for MMP-9 protein expression by Western blotting (bottom, densitometric analysis of bands for MMP-9, relative to α-tubulin normalized to the resting condition). ###,$P < 0.001$ compared with the resting group; **, $P < 0.01$, and ***, $P < 0.001$, compared with the vehicle group.

Statistical Analyses. The experimental results are expressed as the mean ± SEM and are accompanied by the number of observations. For analysis of the results, a one-way analysis of variance (ANOVA) test was performed using Sigma Stat v3.5 software. When group comparisons showed a significant difference, the Student−Newman−Keuls test was used. A P value of <0.05 was considered to be statistically significant.

RESULTS

Effects of Sesamol on MMP-9 Expression and Activation. To investigate the natural compound sesamol in MMP-9 expression and activation, the human chondrosarcoma cell line (SW1353) was exposed to various concentrations of sesamol (5−20 μM) and then stimulated with proinflammatory cytokines such as TNF-α and IL-1β. After stimulation for 24 h, the MMP-9-induced gelatinolysis was concentration-dependently increased by TNF-α. At concentrations of 5, 10, 30, and 50 ng/mL TNF-α could significantly induce gelatinolysis of MMP-9 (1.42 ± 0.06, 1.63 ± 0.03, 1.80 ± 0.04, and 2.04 ± 0.09, respectively) (Figure 1A). Hence, 30 ng/mL TNF-α was chosen as an ideal concentration based on the expression profile of MMP-9. Treatment of SW1353 cells with sesamol (5, 10, and 20 μM) was found to down-regulate TNF-α-mediated MMP-9 gelatinolysis approximated at 1.69 ± 0.04, 1.52 ± 0.08, and 1.38 ± 0.04, respectively (Figure 1B). Furthermore, a marked induction of MMP-9 protein expression (1.71 ± 0.19) by TNF-α (30 ng/mL) was found to be down-regulated in sesamol (5, 10, and 20 μM) pretreated cells; it was noted as 1.6 ± 0.26, 1.35 ± 0.28, and 0.80 ± 0.20, respectively (Figure 1C).

As shown in Figure 2A, the other proinflammatory cytokine, IL-1β, could induce MMP-9-mediated gelatinolysis (1.48 ± 0.07, 1.68 ± 0.09, 1.84 ± 0.02, and 2.02 ± 0.19) in a concentration-dependent manner (5, 10, 30, and 50 ng/mL, respectively). Interestingly, at a concentration of 30 ng/mL, IL-1β mediated gelatinolysis was significantly attenuated by sesamol (5, 10, and 20 μM), and the effect was found to be 1.56 ± 0.05, 1.38 ± 0.07, and 1.30 ± 0.08, respectively (Figure 2B). Consistently, Western blot assay revealed that the same concentration of sesamol exerted a strong inhibition (1.84 ± 0.15, 1.70 ± 0.29, and 1.42 ± 0.21 for 5, 10, and 20 μM, respectively) on IL-1β-induced expression (2.60 ± 0.10) of MMP-9 protein in SW1353 cells (Figure 2C).

To further evaluate whether sesamol inhibits MMP-9 activation in SW1353 cells through cytotoxic effects, cells were preincubated with sesamol (5−20 μM) for 24 h. On the basis of the MTT assay, it was shown that this natural compound had little effect on cell viability of SW1353 cells, even at a higher concentration (20 μM) at about 87.26 ± 1.63% (data not shown).

Effects of Sesamol on MMP-1 and -13 Expressions. To evaluate MMP-1 and -13 expressions in response to PMA followed by the treatment of sesamol, chondrocyte cultures were serum starved for 24 h and then stimulated with PMA (10 ng/mL) in the presence or absence of sesamol (5−20 μM). The results shown in Figures 3 and 4 indicate that, at concentrations of 5, 10, and 20 μM, sesamol could significantly inhibit the activation of MMP-1 (3.01 ± 0.21, 2.58 ± 0.20, and 2.10 ± 0.35, respectively) induced by PMA (3.57 ± 0.38). A similar significantly lower expression (1.48 ± 0.02, 1.08 ± 0.09, and 0.56 ± 0.16) of MMP-13 was noted in sesamol (5, 10, and 20 μM, respectively) pretreated and PMA-induced (1.52 ± 0.12) SW1353 cells. These results indicate that sesamol down-regulates the stimulated expression of MMP-1 and -13 in a

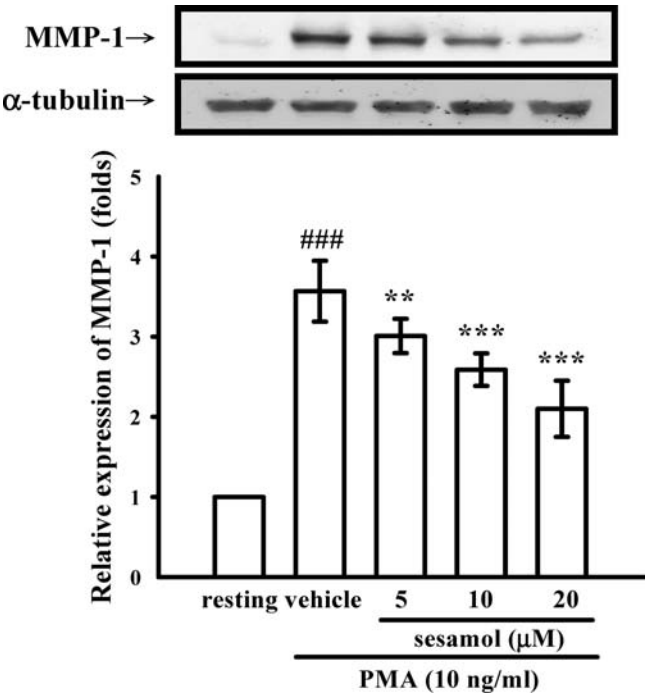

Figure 3. Effects of sesamol on phorbol-12-myristate-13-acetate (PMA)-induced expression of MMP-1 in SW1353 chondrosarcoma cell line. SW1353 (2×10^6) cells were pretreated with the vehicle (DMSO, 0.01%, v/v) or sesamol (5, 10, and 20 μM) and then stimulated by PMA (10 ng/mL) for 24 h. Cell lysates were obtained and analyzed for MMP-1 protein expression by Western blotting (bottom, densitometric analysis of bands for MMP-1, relative to α-tubulin normalized to the resting condition). Data are shown as the mean ± SEM of three independent experiments. ###, $P < 0.001$ compared with the resting group; **, $P < 0.01$, and ***, $P < 0.001$, compared with the vehicle group.

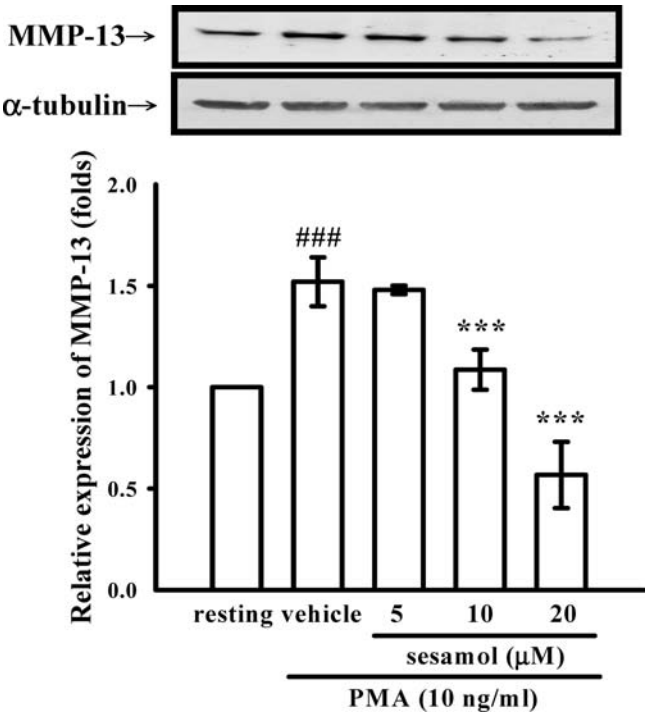

Figure 4. Effects of sesamol on PMA-induced expression of MMP-13 in SW1353 chondrosarcoma cell line. SW1353 (2×10^6) cells were pretreated with the vehicle (DMSO, 0.01%, v/v) or sesamol (5, 10, and 20 μM) and then stimulated by PMA (10 ng/mL) for 24 h. Cell lysates were obtained and analyzed for MMP-13 protein expression by Western blotting (bottom, densitometric analysis of bands for MMP-13, relative to α-tubulin normalized to the resting condition). Data are shown as the mean ± SEM of three independent experiments. ###, $P < 0.001$ compared with the resting group; ***, $P < 0.001$ compared with the vehicle group.

concentration-dependent manner. These MMP-1 and -13 expressions, however, were not enhanced by TNF-α and IL-1β (data not shown).

Sesamol Inhibits TNFα- and PMA-Mediated Degradation of IκB-α. To determine whether the inhibitory action of sesamol was due to an effect on degradation, the cytoplasmic levels of protein were examined by Western blot analysis. As shown in Figure 5, panels A and B, treatment of cells with TNF-α and PMA (for 30 min) could induce degradation rates of about 0.801 ± 0.036 and 0.796 ± 0.023, respectively. Interestingly, SW1353 cells that had been treated with sesamol (5, 10, and 20 μM) for 15 min significantly reversed the rate of degradation (0.92 ± 0.04, 0.096 ± 0.04, and 0.10 ± 0.05, respectively) induced by TNF-α (Figure 5A). The PMA-induced degradation was also effectively reversed by sesamol; it showed 0.92 ± 0.01, 0.98 ± 0.03, and 0.99 ± 0.03 at concentrations of 5, 10, and 20 μM, respectively (Figure 5B). However, the treatment of sesamol did not affect the IL-1β-induced rate of degradation (data not shown).

Sesamol Inhibits TNFα- and PMA-Induced MAPKs. Another possible potent mechanism is an alteration in stimulated intracellular pathway(s) by sesamol. IL-1β has been shown to activate MAPKs (ERK, p38, and JNK) during MMP-1 and -13 productions in SW1353 cells (5, 10, 25). Whereas MMP-1 induction requires the activation of p38 and ERK, MMP-13 induction requires the activation of p38, JNK, and NF-κB.[5] Thus, p38 contributes to the induction of both collagenases in stimulated SW1353 cells. This was compatible with the results of our study that showed that PMA-stimulated the production of MMP-1 and -13 (Figures 3 and 4). To examine whether sesamol affects stimulated ERK, p38, and JNK activations in SW1353 cells, the phosphorylation levels of ERK, p38, and JNK were compared between the treatments with TNF-α and PMA in the presence or absence of sesamol. As shown in Figure 6, PMA (10 ng/mL) was found to significantly stimulate phos-ERK (2.26 ± 0.15) and phos-p38 (3.00 ± 0.02) levels; however, treatment of cells with sesamol (5, 10, and 20 μM) suppressed the PMA-induced levels of phos-ERK (2.16 ± 0.08, 1.54 ± 0.058, and1.25 ± 0.13, respectively) and phos-p38 (2.61 ± 0.09, 2.43 ± 0.09, and1.25 ± 0.10, respectively). Moreover, when the SW1353 cells were preincubated with various concentrations of sesamol (5, 10, and 20 μM) for 15 min, the phos-p38 level was suppressed at about 1.27 ± 0.03, 1.03 ± 0.03, and 0.60 ± 0.05, respectively, from the stimulation (1.84 ± 0.15) with IL1-β (30 ng/mL) for 30 min (Figure 7). Sesamol (5, 10, and 20 μM) also inhibits ERK activation induced by IL1-β (30 ng/mL) in a concentration-dependent manner. However, the presence of sesamol did not affect c-JNK levels (data not shown).

Sesamol Inhibits MMP-1 and -9 Expression in Cartilages of MIA-Induced OA in Rats. MMPs have been implicated in the cartilage matrix degradation associated with both OA and RA. Inhibition of MMPs has long been proposed as a therapeutic modality to prevent the cartilage degradation that occurs as a

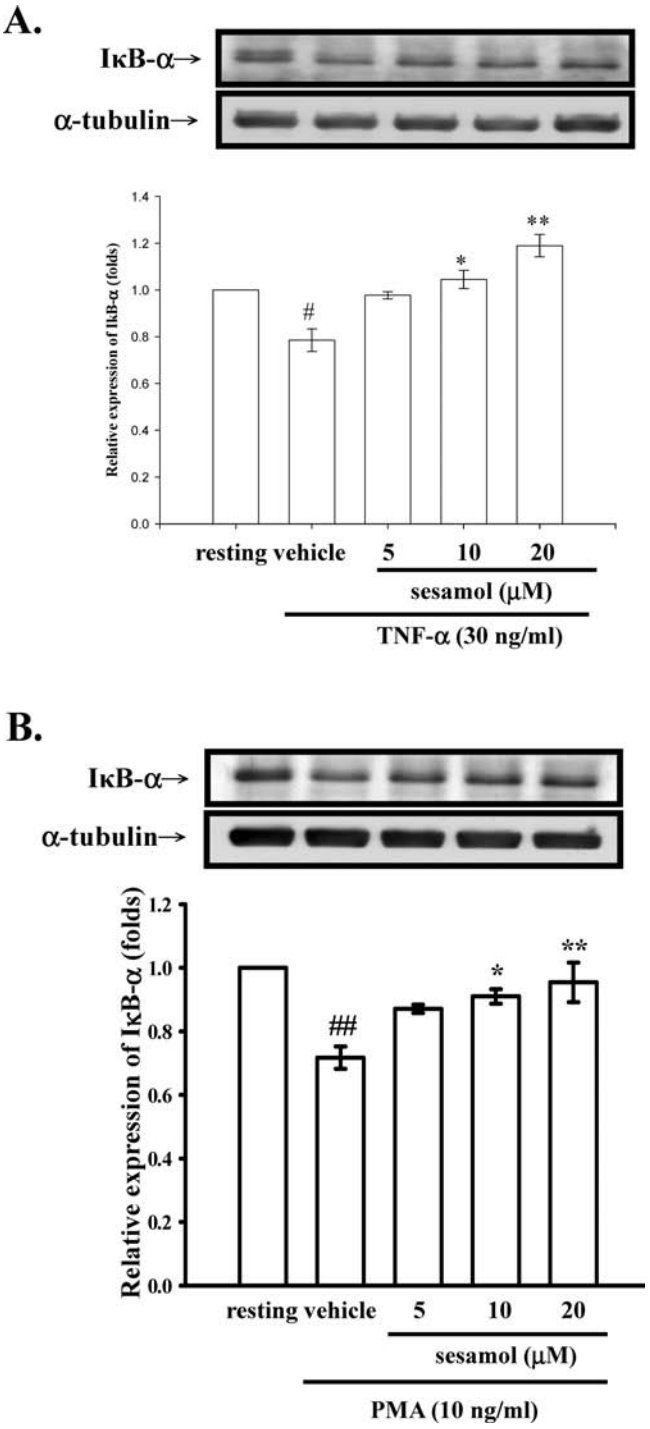

Figure 5. Effects of sesamol on TNF-α- and PMA-induced degradation of IκB-α in chondrosarcoma SW1353 cell lines. Cell lysates were obtained and analyzed for IκB-α protein degradation by Western blotting. SW1353 cells (2×10^6 cells/mL in 6-well plates) were treated with various concentrations of sesamol (5, 10, and 20 μM) or vehicle (DMSO) for 15 min before treatment with TNF-α (A, 30 ng/mL) or PMA (B, 10 ng/mL) for 30 min. α-Tubulin was used as an internal control. The figures are representative examples of three independent experiments. #, $P < 0.05$, and ##, $P < 0.01$, compared with the resting group; ∗, $P < 0.05$, and ∗∗, $P < 0.01$, compared with the vehicle group.

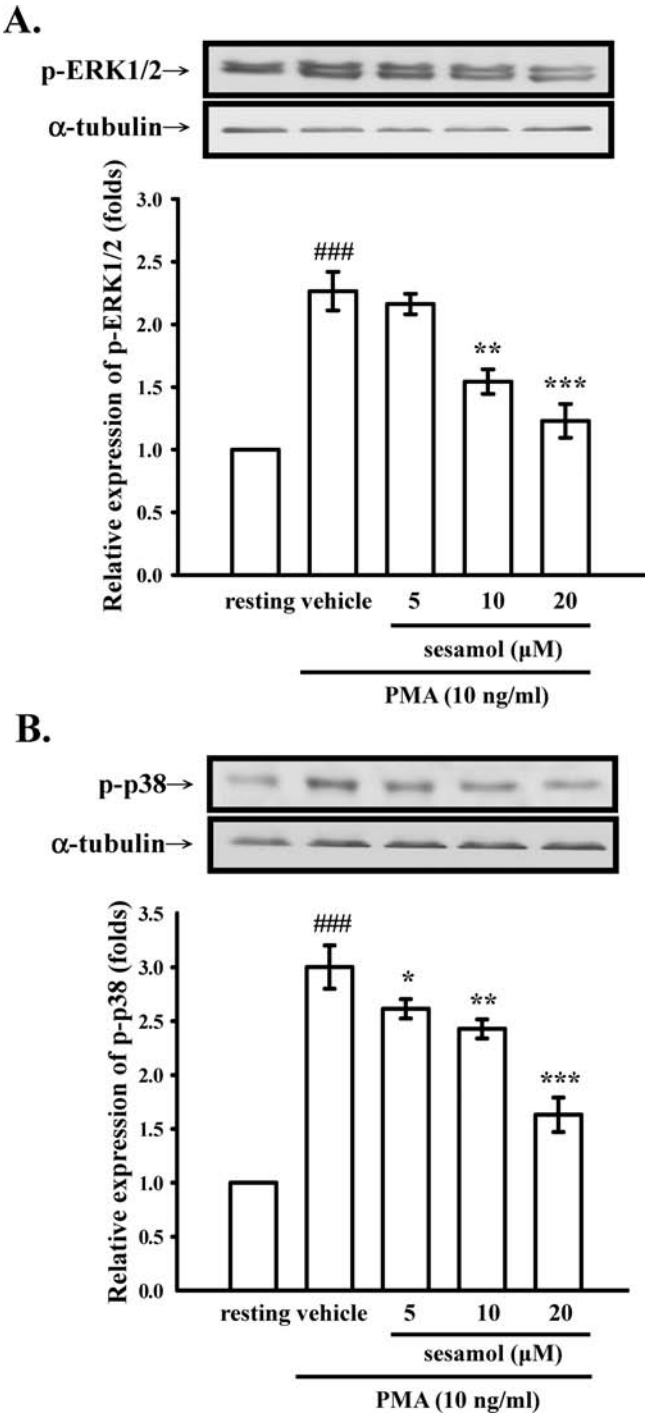

Figure 6. Effects of sesamol on PMA-induced activation of ERK and p38 MAPKs in chondrosarcoma SW1353 cell lines. SW1353 cells (2×10^6 cells/mL in 6-well plates) were treated with various concentrations of sesamol (5, 10, and 20 μM) or vehicle (DMSO) for 15 min before treatment with PMA (10 ng/mL) for 24 h. Cell lysates were obtained and analyzed for phosphorylated (p-) Erk1/2 (A) or p38 (B) MAPK protein expression by Western blotting. α-Tubulin was used as an internal control. The figures are representative examples of three independent experiments. ###, $P < 0.001$ compared with the resting group; ∗, $P < 0.05$, ∗∗, $P < 0.01$, and ∗∗∗, $P < 0.001$, compared with the vehicle group.

result of the arthritic process.[25,26] However, although numerous inhibitors of the MMPs have been projected as potential therapeutic agents, there is lack of information pertaining to the inhibition of MMPs by natural compounds. In the present

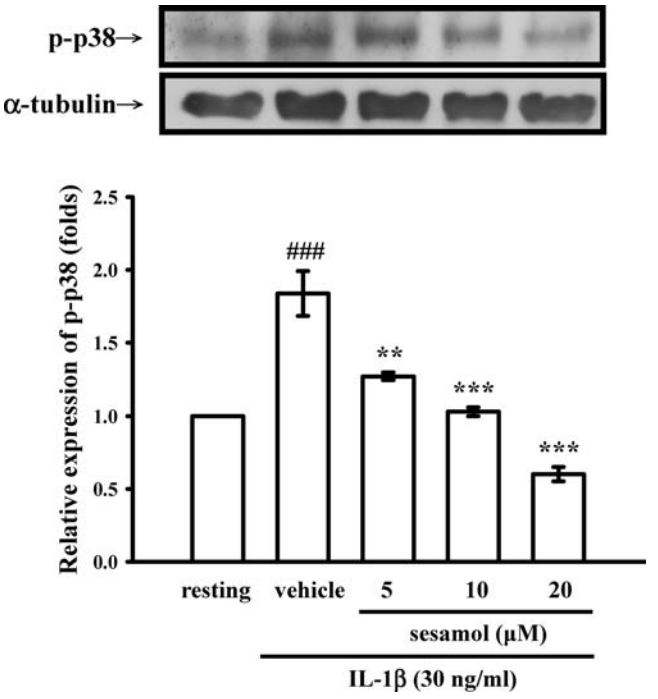

Figure 7. Effects of sesamol on IL-1β-induced activation of p38 MAPK in chondrosarcoma SW1353 cell lines. Cells were treated with various concentrations of sesamol (5, 10, and 20 μM) or vehicle (DMSO) for 15 min before treatment with IL-1β (30 ng/mL). Cell lysates were obtained and analyzed for p-p38 protein expression by Western blotting (bottom, densitometric analysis of bands for p-p38, relative to α-tubulin normalized to the resting condition). ###, $P < 0.001$ compared with the resting group; **, $P < 0.01$, and ***, $P < 0.001$, compared with the vehicle group.

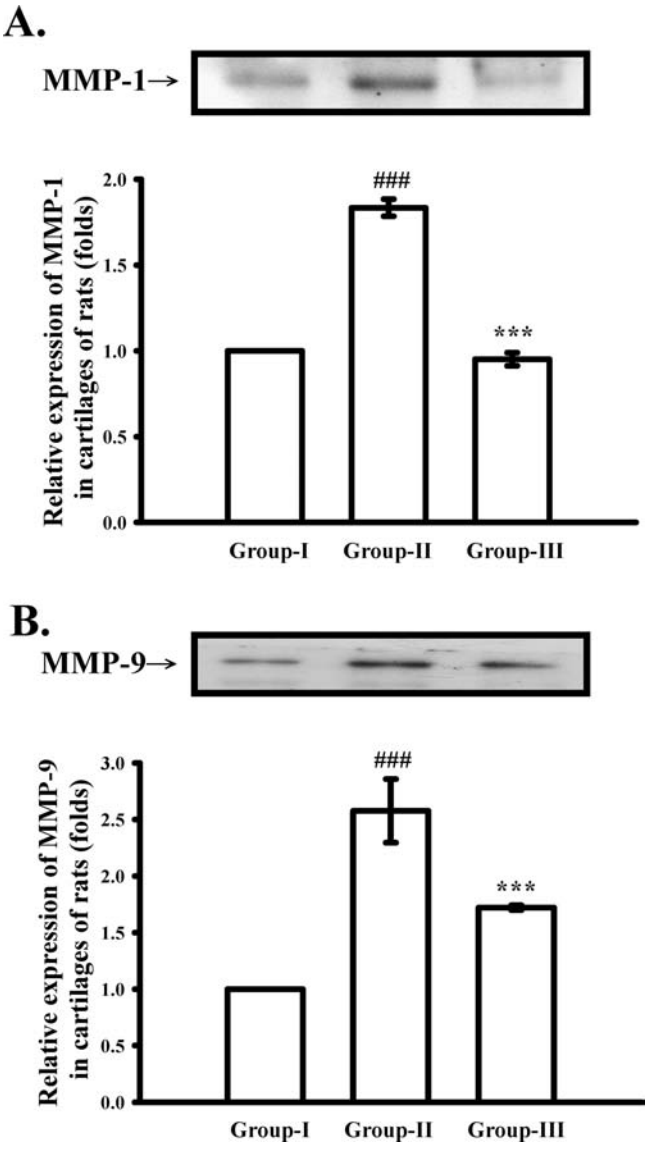

Figure 8. Effects of sesamol on the expression of MMP-1 and -9 of monosodium iodoacetate (MIA)-induced OA rats in vivo. Group I, normal rats; group II, MIA (400 μg/knee) injected rats; group III, MIA (30 mg/kg bw) injected rats that had been treated with sesamol (30 mg/kg bw). Tissue extracts were obtained and analyzed for (A) MMP-1 and (B) MMP-9 protein expression by Western blotting. The figures are representative examples of three independent experiments. ###, $P < 0.001$ compared with the resting group; ***, $P < 0.001$ compared with the vehicle group.

study, sesamol (30 mg/kg bw) was found to be efficacious in inhibiting cartilage damage by suppression of MMP-1 and -9 when dosed after 7 days of MIA treatment (25 μL from 10 mg/mL of MIA dissolved in injectable saline) for 21 days (Figure 8). In MIA-induced untreated rats (group II), significant ($P < 0.001$) increases (relative to normal rats, group I) in the levels of expression of MMP-1 (1.83 ± 0.05) and MMP-9 (2.58 ± 0.28) were observed in the cartilage tissues, respectively (Figure 8). Treatment of MIA-induced (group III) rats with sesamol was found to decrease the mean levels of expression of MMP-1 (0.95 ± 0.04) and MMP-9 (1.72 ± 0.02), so that they approached that of normal rats, presumably by limiting cartilage degradation in the tissues of knee joints. However, MMP-13 cannot be evaluated in a rat model because this species lacks the MMP-13 gene.

DISCUSSION

Much evidence has shown that cartilage chondrocytes were activated to produce MMPs and then decomposition of local connective matrix during the progression of arthritis.[27] Thus, MMPs play an important pathological role, in particular, MMP-1, -9, and -13 as the decomposition factors to induce cartilage damage during inflammation of OA.[28,29] Especially, proinflammatory cytokines such as TNF-α and IL-1β both work as the important stimulating factor in arthritis pathology.[30] Many studies showed that TNF-α may stimulate chondrocytes to produce MMP-1 and -9.[31,32] Our results showed that MMP-9 activation and expression were concentration-dependently induced by TNF-α in SW1353 chondrocytes in a manner similar to that in primary chondrocytes as previously described. After activation of type I TNF-α receptor (TNF-R1), the further through classical NF-κB signaling, activation of IKK, followed by decomposition of IκB-α. Thereafter, the free NF-κB (p65/p66) translocated into the nucleus, binding the corresponding element, to turn on the expression of target genes in chondrocytes.[33] Our findings revealed that sesamol markedly attenuated TNF-α-induced MMP-9 gelatinolysis and production. NF-κB potentially acted as a mediator of IL-1β and TNF-α on chondrocytes for its many the biological effects

(i.e., up-regulation of MMPs, COX-2, and inducible nitric oxide synthase).[34,35] NF-κB also has shown to regulate the expression of adhesion molecules such as cell adhesion molecule-I (I-CAM), vascular endothelial growth factor (VEGF), urokinase plasminogen activator (uPa), Bcl-2, and proinflammatory cytokines in other cell types.[36,37] The mechanism of down-regulation of chondrocyte-specific matrix synthesis may also involve NF-κB activation.[38] It is apparent that OA synovial tissue has been considered as a typical marker of NF-κB,[39] and the synthesis of inflammatory and destructive mediators from OA synovial tissue was reported to be NF-κB dependent.[40] It has been proposed that inhibited NF-κB (p65/p66) transactivation for abrogating TNF-α signaling results from either a decrease in phosphorylation or a reduction in the degradation of IκB-α. We found that the degradation was significantly inhibited by sesamol. These results were similar to the inhibitory capacities of sesamin in human aortic endothelial cells.[34]

On the other hand, it was found that IL-1β could stimulate chondrocytes to produce MMP-9. The results revealed that sesamol significantly inhibited IL-1β-induced MMP-9 gelatinolysis and its production. It is well-known that IL-1β-induced activation by its receptor, through specific adaptors and MEKK, downstream kinases such as MAPKs, and activating factor-1 were sequentially activated.[35] Both ERK and p38 MAPK activations were involved in the process of stimulation of MMP-9 generation by chondrocytes.[36] Similarly, activation of p38 MAPK by IL-1β is also important to stimulate the process of the signaling on MMP expression.[37,38] Therefore, we investigated the role of p38 MAPK pathway on the inhibition of IL-1β-induced MMP expression by sesamol. The results revealed that sesamol markedly attenuated IL-1β-induced phosphorylation of p38 MAPK. These results were consistent with the cartilage breakdown study, which revealed that cytokine increased chondrocyte MMP expression through a p38 MAPK-mediated pathway.[39]

Furthermore, PKC activator such as phorbol ester could simulate some pathological stimulations through activating various signaling pathways in chondrocytes.[40,41] In addition, it was found PMA induced MMP-1 and -13 expressions in the SW1353 chondrocytes as demonstrated in a previous study.[9] It was found that treatment of sesamol significantly inhibited PMA-induced MMP-1 and -13 productions. Under PKC stimulation, the signalings of NF-κB and MAPKs were activated, respectively.[40] Therefore, inhibition of NF-κB attenuated PMA-induced production of inflammatory mediators such as MMP and iNOS.[42,43] Similarly, the stability was restored by sesamol. It was proposed that sesamol exerted attenuation of NF-κB activation through inhibition on TNF-α- and PMA-stimulated IκB-α degradation in human chondrocytes. Consistently, it was mentioned that sesamol could attenuate NF-κB activation through the upstream signaling inhibition on p65 translocation and IκB-α degradation, following reduction of the production of iNOS in mouse macrophages.[44] Furthermore, this natural compound also attenuated PMA-stimulated phosphorylation of ERK1/2 and p38 MAPK, respectively. These results were consistent with treatment of p38 MAPK, or ERK inhibition could reduce the production of PMA-induced MMPs in different human cells.[45,46] Particularly, the inhibition action of sesamol was on the ERK and p38 MAPK pathway, but there was no significant effect on that of JNK MAPK.

OA is a complicated disease, and its pathogenesis is not fully understood. The animal model of intraarticular injected MIA OA mimics the phenotype of human OA such as the inflammatory response and cartilage degeneration.[22,47] In addition, the MIA-induced OA animal model was useful for the exploration of the efficacy and mechanism of therapeutic agents in OA.[48,49] However, it was reported that there is no full correlation between MIA-induced OA in rats and human OA in both transcriptional profilings of arthritic cartilages.[50] MIA is known to induce MMP production during the progression of OA, resulting in induction of cartilage matrix destruction.[22,51] Consistently, this study showed that intraarticular injection of MIA markedly induced MMP-1 and -9 expressions in cartilage extract. Furthermore, treatment of sesamol prevented MMP-1 and -9 expressions in MIA-induced OA in rats. It was reported that inhibition of the activity or production on MMPs could reduce cartilage injury of OA.[52,53] Indeed, the present study found that bioactive sesamol could reduce the elevated expression of MMP-1/-9 in MIA-induced OA, although we did not examine the cartilage destruction and inhibitory effects of sesamol.

In conclusion, this study showed that seasamol attenuated MMP-9, -1, and -13 expression in TNF-α-, IL-1β- or PMA-stimulated chondrocytes and that these effects might be mediated through inhibition of NF-κB and ERK/p38 MAPK activation, respectively. In addition, sesamol significantly inhibited MMP expression on the cartilage of MIA-induced OA rats in vivo. These results could provide new potential opportunities for the development of therapeutics on destructive joint diseases.

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Notes

We declare that there are no conflicts of interest.

ABBREVIATIONS USED

MMPs, matrix metalloproteinases; OA, osteoarthritis; RA, rheumatoid arthritis; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; PMA, phorbol 12-myristate 13-acetate; ERK, extracellular signal-regulated kinase,; cJNK, c-Jun N-terminal kinase; MIA, monosodium iodoacetate.

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