

Chlorogenic acid prevents inflammatory responses in IL-1 β -stimulated human SW-1353 chondrocytes, a model for osteoarthritis

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Abstract: Chlorogenic acid (CGA), which is a natural compound found in various plants, has been reported to exert notable anti-inflammatory activities. The present study investigated the effects and underlying mechanism of CGA on interleukin (IL)-1 β -induced osteoarthritis (OA) chondrocytes. An *in vitro* OA-like chondrocyte model was established using IL-1 β -stimulated human SW-1353 chondrocytes. Cell viability was assessed using an MTT assay. Nitric oxide (NO) and IL-6 production were evaluated by Griess reaction and ELISA, respectively. The expression levels of inducible nitric oxide synthase (iNOS), prostaglandin E₂ (PGE₂), cyclooxygenase 2 (COX-2), collagen II, matrix metalloproteinase (MMP)-13, p65 nuclear factor (NF)- κ B and inhibitor- κ B α were detected by western blot analysis. The results indicated that CGA reversed IL-1 β -induced increases in iNOS/NO, IL-6, MMP-13 and COX-2/PGE₂ production, and reversed the IL-1 β -mediated downregulation of collagen II. In addition, the data suggested that CGA was capable of inhibiting the IL-1 β -induced inflammatory response, at least partially via the NF- κ B signaling pathway. In conclusion, CGA may be considered a suitable candidate agent in the treatment of OA.

Introduction

Osteoarthritis (OA) is a common disease of the elderly worldwide, which is characterized by articular cartilage destruction and local inflammation, resulting in pain, disability and a significantly reduced quality of life for the affected individuals (1). Accumulating evidence (2,3) has indicated that

low-grade systemic and local inflammation is involved in the progression of OA (4). Several inflammatory mediators, such as interleukin (IL)-1 β , nitric oxide (NO), prostaglandin E₂ (PGE₂) and fragments of cartilage proteins including matrix metalloproteinases (MMPs), have been reported to be elevated in patients with OA (5). IL-1 β is an important proinflammatory cytokine during the inflammatory response, which has been reported to serve critical roles in joint inflammation and cartilage destruction processes. For example, IL-1 β elevates NO production, improves cyclooxygenase-2 (COX-2) activity and upregulates the expression of MMPs, including MMP-13, which is associated with extracellular matrix (ECM) degradation in cartilage (6,7). Based on the significant role of inflammation in OA, anti-inflammatory treatment may have significant value in the treatment of OA.

Previous research has also indicated that IL-1 β , or other certain stimuli including tumor necrosis factor (TNF)- α or lipopolysaccharide (LPS), may activate nuclear factor (NF)- κ B (8), which regulates the expression of several other proinflammatory cytokines and proteases, and mediates critical events in the inflammatory responses of chondrocytes (9). Under normal physiological circumstances, NF- κ B is sequestered and bound to its inhibitory protein, inhibitor- κ B α (I κ B α). When inflammation is induced, I κ B α undergoes proteasomal degradation. This event is followed by translocation of cytoplasmic NF- κ B into the nucleus, where it activates transcription of numerous proinflammatory cytokines, adhesion molecules and inflammatory mediators, such as inducible nitric oxide synthase (iNOS), TNF- α and MMPs.

Chlorogenic acid (CGA; Fig. 1) is formed from the esterification of caffeic acid and quinic acid, and is a polyphenolic compound isolated from numerous plants, including coffee beans (10), *Lonicerae japonicae* (11) and *Eucommiae cortex* (12). Previous studies based on *in vivo* and *in vitro* systems have revealed that CGA exerts antioxidant (13), antibacterial (14) and antitumor activities (15). Furthermore, CGA has demonstrated protective effects against several experimental disorders, including mastitis (16), gastroesophageal reflux disease (17) and LPS-induced acute lung injury (18) via downregulation of the inflammatory response. These findings suggested that CGA may serve as a novel therapeutic agent

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for the treatment of inflammatory diseases. Notably, *in vitro* studies have demonstrated that CGA attenuates the production of several inflammatory mediators, including IL-1 β , IL-6 and TNF- α in LPS-stimulated RAW264.7 cells (19). In addition, Lee *et al* demonstrated the anticandidal and anti-arthritis effects of CGA by inhibiting the synthesis of NO in LPS-activated macrophages (20). CGA may also decrease osteoclast differentiation and bone resorption *in vivo* and may have therapeutic potential in treating diseases associated with inflammatory bone destruction (21). Studies have addressed the therapeutic potential of CGA on arthritis; however, its ability to protect against IL-1 β -induced OA remains poorly understood.

Human SW-1353 chondrosarcoma cells stimulated with IL-1 β possess similar properties to the articular chondrocytes of OA (6). The present study employed this OA-like chondrocyte cellular model as an *in vitro* tool, in order to investigate the effects of CGA on the expression levels of iNOS/NO, IL-6, COX-2/PGE₂, collagen II/MMP-13 and NF- κ B signaling pathways. The results indicated that CGA may be of clinical value in the treatment of OA.

Materials and methods

Chemicals and reagents. CGA (purity >98%) was purchased from Beijing Dewei Sodium Biotechnology Co., Ltd. (Beijing, China) and confirmed by high-performance liquid chromatography by the Research and Engineering Center for Natural Medicine, Xi'an Jiaotong University (Xi'an, China). L-15 medium was obtained from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Fetal bovine serum was purchased from Hyclone (GE Healthcare Life Sciences, Logan, UT, USA). Trypsin was purchased from Amresco, LLC (Solon, OH, USA). IL-1 β was purchased from PeproTech, Inc. (Rocky Hill, NJ, USA). Ethylenediamine tetra-acetic acid (EDTA) and 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Griess reagent was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kit for human IL-6 (cat. no. D6050) was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Antibodies against COX-2 (cat. no. 12375-1-AP), MMP-13 (cat. no. 18165-1-AP), collagen II (cat. no. 15943-1-AP), NF- κ B (cat. no. 10745-1-AP) and I κ B- α (cat. no. 10268-1-AP) were purchased from ProteinTech, Inc. (Rosemont, IL, USA). Antibodies against phosphorylated (p)-NF- κ B (cat. no. 3033) and p-I κ B- α (cat. no. 2859) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). PGE₂ rabbit polyclonal antibody (cat. no. ab2318) and iNOS rabbit monoclonal antibody (cat. no. ab178945) were purchased from Abcam (Cambridge, MA, USA). Rabbit anti-GAPDH (cat. no. PA1-987), bicinchoninic acid (BCA) protein assay reagent kit and Pierce enhanced chemiluminescent (ECL) Plus Substrate were purchased from Pierce (Thermo Fisher Scientific, Inc.).

Cell culture. Human SW-1353 chondrocytes were purchased from the Shanghai Institute of Cell Biology, the Chinese Academy of Sciences (Shanghai, China) and cultured in L-15 supplemented with 10% fetal bovine serum, 100 U/ml

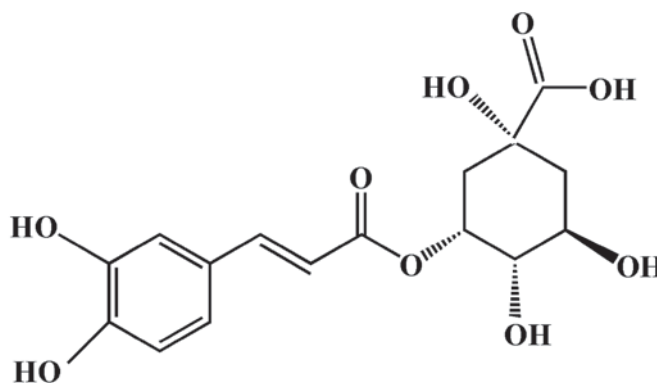


Figure 1. Chemical structure of chlorogenic acid.

penicillin and, 100 μ g/ml streptomycin. Cells were incubated in a humidified incubator in an atmosphere containing 5% CO₂ at 37°C. The culture medium was replaced every 3 days.

Cell viability assay. To evaluate the cytotoxic effects of CGA, cell viability was assessed using the MTT assay. Exponentially growing SW-1353 cells were plated into 96-well plates (2 \times 10⁶ cells/ml) and cultivated overnight. The following day, CGA (31.25, 62.5, 125, 250, 500 or 1,000 μ g/ml), or various concentrations of CGA along with IL-1 β (10 ng/ml), was added to the cells, which were incubated for 48 h at 37°C. The control cells received an equal amount of dimethyl sulfoxide (DMSO). Following removal of the medium, 180 μ l serum-free medium and 20 μ l MTT (5 mg/ml) was added to each well, and the plates were incubated at 37°C for 4 h. The supernatants were replaced with 150 μ l DMSO to dissolve the crystals, the plates were agitated for 10 min, and the absorbance was measured at 490 nm using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Survival was calculated according to the formula: Survival=OD_{test}/OD_{control}, where OD indicates optical density.

NO assay. NO production was assessed spectrophotometrically via the determination of nitrite concentrations in the cell culture supernatant using Griess reagent. SW-1353 chondrocytes (2 \times 10⁶ cell/ml) were pretreated with various concentrations of CGA (50, 100, 200 or 500 μ g/ml) for 24 h, and were subsequently exposed to IL-1 β (10 ng/ml) for 24 h at 37°C. After 24 h the supernatants were collected. Each supernatant was mixed with an equal volume of Griess reagent (150 μ l) and incubated for 5 min at room temperature. Absorbance was subsequently determined at 540 nm. Sodium nitrite was used to prepare the standard curve.

ELISA detection of IL-6 production. SW-1353 chondrocytes (2 \times 10⁶ cell/ml) were pretreated with various concentrations of CGA (50, 100, 200 or 500 μ g/ml) for 24 h, after which the cells were stimulated with IL-1 β (10 ng/ml) for 24 h at 37°C. The amount of IL-6 secreted into the culture medium was measured using a commercially available IL-6-specific ELISA kit according to the manufacturer's protocol.

Western blot analysis. SW-1353 chondrocytes (2 \times 10⁶ cells/ml) were seeded and cultured in 6-well plates. The cells were

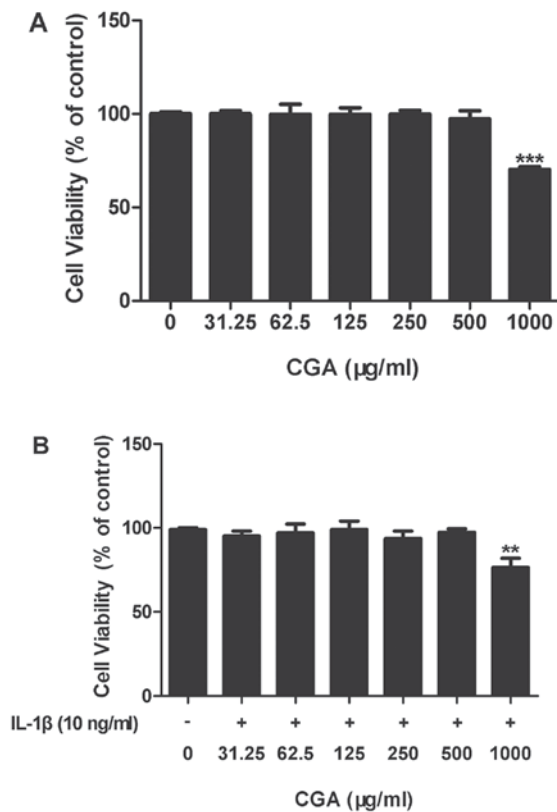


Figure 2. Effects of CGA and/or IL-1 β on the viability of human SW-1353 chondrocytes. (A) Chondrocytes were treated with CGA (31.25, 62.5, 125, 250, 500, or 1,000 μ g/ml) alone for 48 h. (B) Chondrocytes were treated with CGA (31.25, 62.5, 125, 250, 500 or 1,000 μ g/ml) and IL-1 β (10 ng/ml) for 48 h. Values are expressed as the mean \pm standard error of the mean (n=3). **P<0.01; ***P<0.001 vs. the control group. CGA, chlorogenic acid; IL, interleukin.

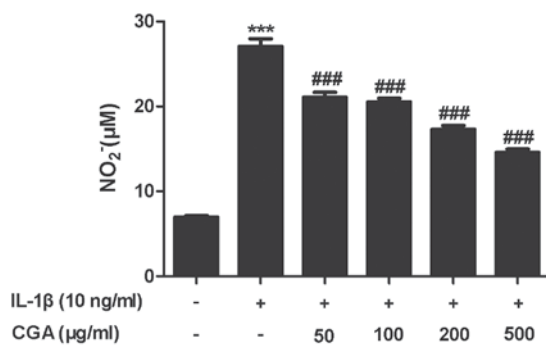


Figure 3. Effects of CGA on IL-1 β -induced NO production in human SW-1353 chondrocytes. Chondrocytes were pretreated with CGA (50, 100, 200 or 500 μ g/ml) for 24 h followed by exposure to IL-1 β (10 ng/ml) for 24 h. NO production was assessed by the Griess reaction. Values are presented as the mean \pm standard error of the mean. ***P<0.05 vs. the control group; ###P<0.001 vs. the IL-1 β alone group. CGA, chlorogenic acid; IL, interleukin; NO, nitric oxide.

pretreated with CGA (50 or 200 μ g/ml) for 24 h at 37°C, after which the cells were exposed to IL-1 β (10 ng/ml) for 30 min. Total proteins were isolated using lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 20 mM NaF, 0.5% NP-40, 1% Triton, 1% protease inhibitor and phosphatase inhibitor cocktail]. Concentrations were determined using a BCA Protein

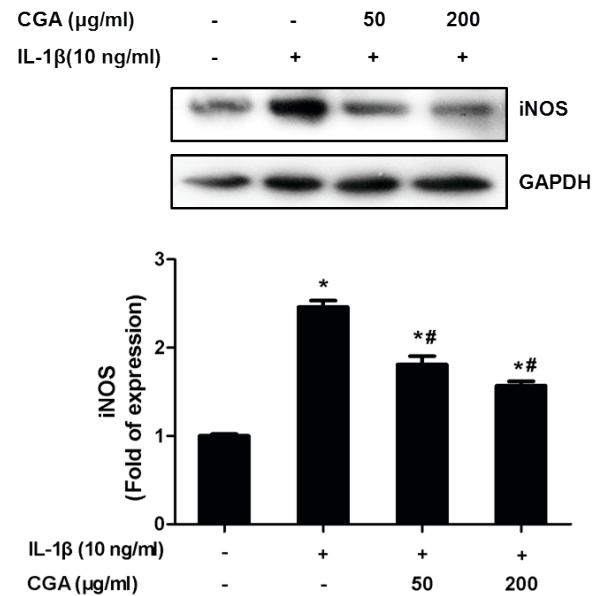


Figure 4. Effects of CGA on IL-1 β -induced protein expression of iNOS in human SW-1353 chondrocytes. Chondrocytes were pretreated with CGA (50 or 200 μ g/ml) for 24 h, followed by incubation with IL-1 β (10 ng/ml) for 30 min. The expression of iNOS was determined by western blot analysis. The data represents band intensity compared with untreated samples, GAPDH was used for normalization. Data are presented as the mean \pm standard error of the mean. *P<0.05 vs. the control group; #P<0.05 vs. the IL-1 β alone group. CGA, chlorogenic acid; IL, interleukin; iNOS, inducible nitric oxide synthase.

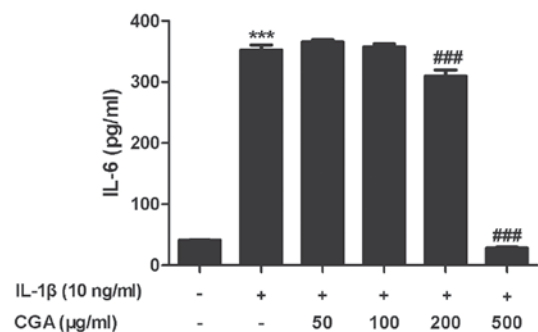


Figure 5. Effects of CGA on the levels of IL-6 from IL-1 β -stimulated SW-1353 chondrocytes. Cells were incubated with CGA (50, 100, 200 or 500 μ g/ml) for 24 h prior to exposure to IL-1 β (10 ng/ml) for 24 h. The levels of IL-6 were measured by ELISA. Data are presented as the mean \pm standard error of the mean. ***P<0.05 vs. the control group; ###P<0.001 vs. the IL-1 β alone group. CGA, chlorogenic acid; IL, interleukin.

Quantification kit. The proteins (30 μ g/sample) were then separated on different percentages of SDS-polyacrylamide gel (6-10%) and were transferred onto polyvinylidene difluoride membranes. Nonspecific sites were blocked with Tris-buffered saline 0.1% Tween-20 (TBST) plus 5% nonfat dry milk for 2 h at room temperature, and the membranes were then incubated with primary antibodies (1:1,000 dilution) in 5% skim milk overnight at 4°C. The blots were subsequently washed three times with TBST, and incubated with horseradish peroxidase-linked secondary antibodies (1:15,000 dilution; cat. no. 31463; Pierce; Thermo Fisher Scientific, Inc.) for 2 h

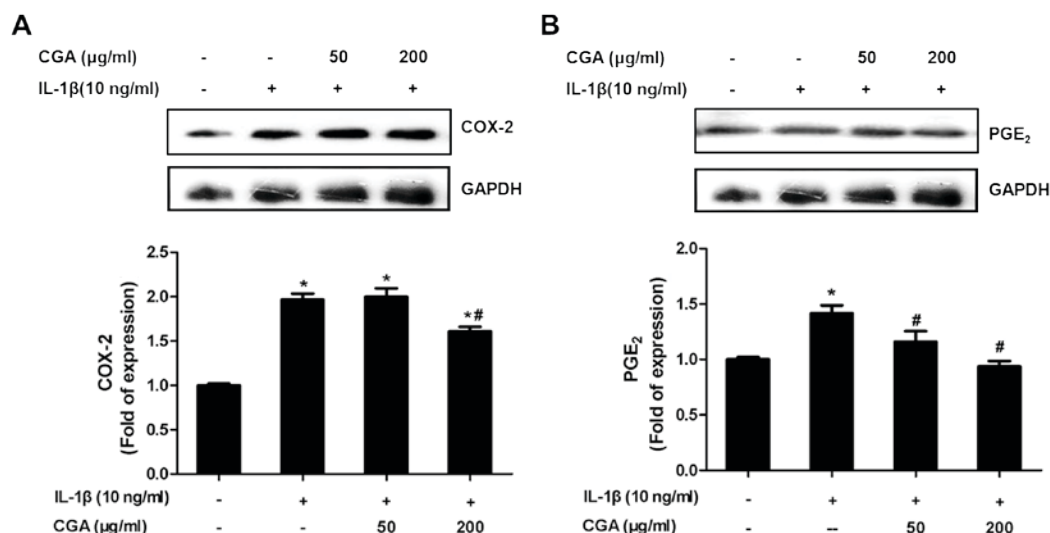


Figure 6. Effects of CGA on the expression of COX-2 and PGE₂ from human SW-1353 chondrocytes. Chondrocytes (2×10^6 cells/ml) were treated with CGA (50 or 200 μ g/ml) for 24 h followed by IL-1 β (10 ng/ml) treatment for 30 min. The expression levels of (A) COX-2 and (B) PGE₂ were determined by western blot analysis. Data are presented as the mean \pm standard error of the mean. * $P < 0.05$ vs. the control group; # $P < 0.05$ vs. the IL-1 β alone group. CGA, chlorogenic acid; IL, interleukin; COX-2, cyclooxygenase 2; PGE₂, prostaglandin E₂.

at room temperature, and washed three further times with TBST. Protein bands were detected using a Pierce ECL Plus Substrate. GAPDH band intensity was used as internal control to normalize protein expression levels. The optical density was measured using Quantity One 1-D Analysis software (version 4.4; Bio-Rad Laboratories, Inc.).

Statistical analysis. All results were presented as the mean \pm standard error of the mean of ≥ 3 independent experiments for each test. Differences among the various groups were assessed by one-way analysis of variance followed by Dunnett's test. Statistical analyses were performed using SPSS v. 12.0 software (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of CGA and/or IL-1 β on human SW-1353 chondrocyte cell viability. An MTT assay was used to determine the effects of CGA on the cell viability of IL-1 β -induced human SW-1353 chondrocytes. The results indicated that CGA was cytotoxic to human SW-1353 chondrocytes at a concentration of 1,000 μ g/ml; however, cell viability in cells treated with up to 500 μ g/ml CGA was not significantly different from the control cells, with or without IL-1 β stimulation (Fig. 2). Based on these results, the following experiments were conducted using CGA at concentrations ≤ 500 μ g/ml.

Effects of CGA on IL-1 β -stimulated NO production and iNOS expression in human SW-1353 chondrocytes. NO serves a pivotal role in OA progression. The results of the viability assay indicated that treatment with 500 μ g/ml CGA for 48 h was not cytotoxic; therefore, the inhibitory effects of CGA (50, 100, 200 and 500 μ g/ml) on NO release in stimulated SW-1353 chondrocytes were examined. NO production was significantly enhanced following IL-1 β activation for 24 h,

and pretreatment with CGA significantly diminished the IL-1 β -induced NO release in a concentration-dependent manner (Fig. 3). NO synthesis is predominantly catalyzed by iNOS; therefore, iNOS expression in SW-1353 chondrocytes was investigated. Consistent with the aforementioned results, CGA was able to inhibit IL-1 β -induced iNOS protein expression (Fig. 4).

Effects of CGA on the expression of IL-6 in IL-1 β -stimulated SW-1353 chondrocytes. To evaluate the effects of CGA on IL-6 production in human SW-1353 chondrocytes, IL-6 secretion was examined by ELISA. Human SW-1353 chondrocytes were pretreated with CGA for 24 h and then stimulated with IL-1 β for 24 h. IL-6 secretion from human SW-1353 chondrocytes was stimulated by IL-1 β treatment, and this was reduced by treatment with CGA (Fig. 5). The observed alterations occurred in a concentration-dependent manner.

Inhibitory effects of CGA on COX-2 and PGE₂ expression in IL-1 β -stimulated SW-1353 chondrocytes. To evaluate whether CGA affected the levels of COX-2 and PGE₂ in IL-1 β -induced SW-1353 chondrocytes, the protein expression levels of COX-2 and PGE₂ were investigated by western blot analysis (Fig. 6). The results indicated that CGA significantly diminished IL-1 β -stimulated expression of COX-2 and PGE₂, thus suggesting that CGA may inhibit the synthesis of PGE₂ by influencing the enzymatic activity of COX-2.

Inhibitory effects of CGA on collagen II and MMP-13 expression in IL-1 β -stimulated SW-1353 chondrocytes. Protein expression levels of collagen II and MMP-13 were examined using western blot analysis (Fig. 7). As expected, CGA significantly diminished IL-1 β -induced MMP-13 protein expression, and markedly reversed the downregulation of collagen II expression in IL-1 β -stimulated SW-1353 chondrocytes.

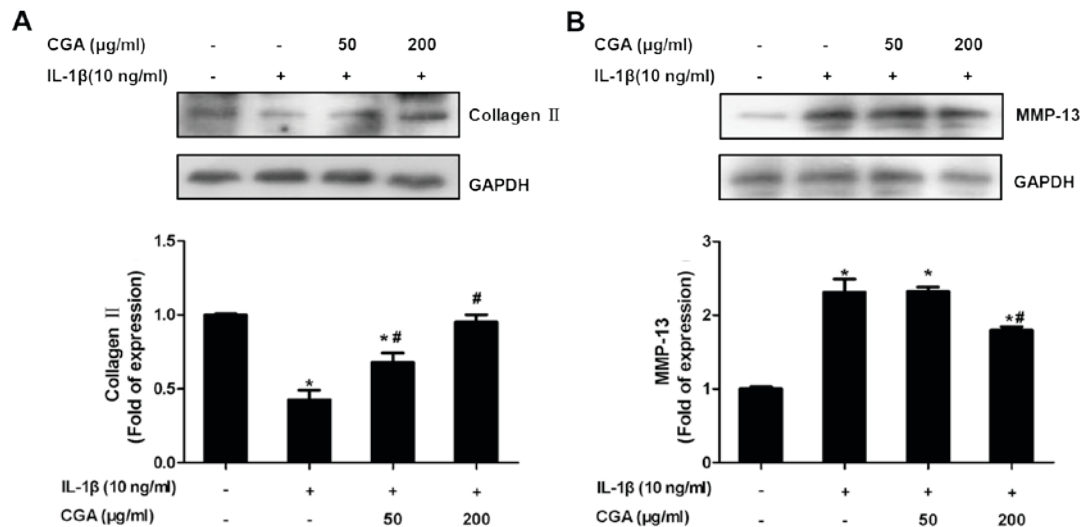


Figure 7. Effects of CGA on the expression of collagen II and MMP-13 from IL-1 β -stimulated SW-1353 chondrocytes. Chondrocytes (2×10^6 cells/ml) were treated with CGA (50 or 200 μ g/ml) for 24 h, and IL-1 β (10 ng/ml) was added for 30 min. The expression levels of (A) collagen II and (B) MMP-13 were analyzed by western blot analysis. Data are presented as the mean \pm standard error of the mean. * $P < 0.05$ vs. the control group; # $P < 0.05$ vs. the IL-1 β alone group. CGA, chlorogenic acid; IL, interleukin; MMP, matrix metalloproteinase.

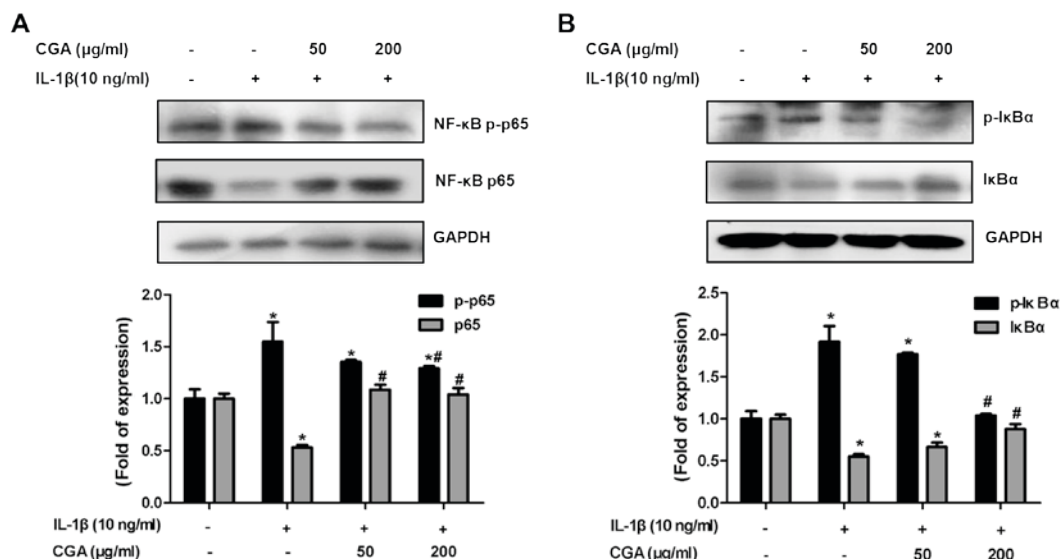


Figure 8. Effects of CGA on the I κ B α /NF- κ B signaling pathway from IL-1 β -stimulated SW-1353 chondrocytes. Chondrocytes (2×10^6 cells/ml) were pretreated with CGA (50 or 200 μ g/ml) for 24 h, followed by incubation with IL-1 β (10 ng/ml) for 30 min. Protein levels of (A) NF- κ B p65 and NF- κ B p-p65 and (B) I κ B α and p-I κ B α were examined by western blot analysis. Data are presented as the mean \pm standard error of the mean. * $P < 0.05$ vs. the control group; # $P < 0.05$ vs. the IL-1 β alone group. CGA, chlorogenic acid; IL, interleukin; NF- κ B, nuclear factor κ B; I κ B α , inhibitor- κ B α ; p, phosphorylated.

Effects of CGA on the I κ B α /NF- κ B signaling pathway in IL-1 β -stimulated SW-1353 chondrocytes. The NF- κ B signal transduction pathway serves vital roles in cartilage degradation and the inflammatory response. To investigate the effects of CGA-mediated inflammatory response inhibition on IL-1 β -stimulated chondrocytes, NF- κ B and its inhibitory protein I κ B α were detected. Western blot analysis was used to detect the levels of NF- κ B p65 phosphorylation and I κ B degradation. IL-1 β stimulation resulted in the phosphorylation of I κ B α and NF- κ B p65, whereas CGA reversed this effect (Fig. 8). These results indicated that the NF- κ B signal transduction pathway may be inhibited by CGA in IL-1 β -stimulated SW-1353 chondrocytes.

Discussion

OA is the most prevalent joint disease, and there are currently no effective treatments to limit disease progression. Inflammation has been recognized as an important process in the pathogenesis of OA. Proinflammatory cytokines, including TNF- α and IL-1, become activated during OA progression, and this induces inflammatory responses and cartilage destruction (3). Commonly-used pharmacological treatments, such as oral non-steroidal anti-inflammatory drugs, for the treatment of patients with OA, are often associated with serious adverse effects (22). Therefore, plant-derived agents that possess anti-inflammatory activity and low toxicity are

more frequently being employed as a therapeutic option in OA (23). CGA, which is a bioactive phenolic acid component isolated from plants, displays anti-inflammatory activity and has been recommended for the treatment of OA (24). The present study aimed to determine the anti-inflammatory and cartilage-protective effects of CGA on IL-1 β -stimulated SW-1353 chondrocytes.

IL-1 β is an important proinflammatory cytokine released by activated synoviocytes or chondrocytes, which serves an important role in the progression of OA (25). Several studies have indicated that IL-1 β upregulates NO production via stimulation of iNOS activity, and elevates the COX2-mediated expression of PGE₂. In addition, IL-1 β induces the expression of other proinflammatory cytokines, including IL-6 and IL-17, and stimulates matrix remodeling via the regulation of MMPs, resulting in the degradation of aggrecan and other matrix constituents (26,27). Therefore, IL-1 β -stimulated SW-1353 cells have been employed as an *in vitro* cellular model of OA (8,28).

NO is produced from the amino acid L-arginine by the enzymatic action of iNOS, which is induced by inflammatory stimuli, indicating that this proinflammatory mediator could contribute to OA disease (29). In the present study, statistical analysis revealed that CGA markedly decreased iNOS expression and NO production from IL-1 β -stimulated SW-1353 chondrocytes in a concentration-dependent manner, thus suggesting that CGA may possess an anti-inflammatory role via the regulation of NO synthesis.

PGE₂ is another key inflammatory mediator; PGE₂ is synthesized from arachidonic acid via the stimulation of COX-2 during the inflammatory response. COX-2 has been recognized as an inducible or pathological enzyme that participates in the inflammatory response by facilitating PGE₂ generation, and promotes the inflammatory cytokine-induced metabolic imbalance of cartilage proteoglycans, thereby advancing arthritis (30,31). IL-6 has been considered as another crucial cytokine involved in the inflammatory response. The present study explored the anti-inflammatory role of CGA, and the results indicated that CGA may inhibit the protein expression of PGE₂ and COX-2, and the secretion of IL-6, in human SW-1353 chondrocytes in a concentration-dependent manner.

Cartilage destruction is a fundamental disease-progression process that occurs in OA. Therefore, the role CGA serves in abrogating this process was further explored. Chondrocytes are the solitary cellular components of cartilage; under normal conditions, these cells maintain the ECM, and balance anabolic and catabolic metabolism (32). The human SW-1353 chondrosarcoma line has been employed in several studies as an *in vitro* model to investigate the molecular mechanisms and pharmacological treatments for OA, due to its similarity with human OA chondrocytes (7,33). MMPs are a family of zinc-dependent proteases that serve important roles in cartilage ECM degradation and maintenance, and are upregulated in pathophysiological inflammatory conditions (34). MMP upregulation results in the erosion of articular cartilage and thus participates in OA progression. In particular, the collagenase MMP-13 preferentially cleaves type II collagen, which is the predominant collagen involved in the structural and functional integrity of cartilage (35). The present study explored whether CGA was able to exert chondroprotective effects via MMP suppression in chondrocytes. The results indicated that

CGA reduced MMP-13 expression and reversed the reduction of collagen II induced by IL-1 β , compared with the untreated group from human SW-1353 chondrocytes.

NF- κ B is a cytokine-induced transcription factor that serves an important role in regulating the expression of various genes, including numerous proinflammatory cytokines, adhesion molecules and proteases in arthritis, such as MMPs, PGE₂, NO and IL-6 (9); several of the IL-1 β -induced inflammatory mediators in chondrocytes are regulated by the NF- κ B signaling pathway (36). In light of the role of NF- κ B in OA progression, blocking its activity may have therapeutic advantages. The p65 subunit of NF- κ B is normally sequestered into the cytoplasm under resting conditions, and is blocked by I κ B α proteins (37). During inflammation, I κ B α proteins are phosphorylated and degraded by the proteasome, the NF- κ B p65 subunit is released and phosphorylated and then translocated into the nucleus, where it binds to DNA promoter regions and activates the transcription of target genes (38). In the present study, CGA intervention was able to reduce I κ B α degradation and consequently decrease phosphorylated-NF- κ B p65 levels in human SW-1353 chondrocytes, and this occurred in a concentration-dependent manner. These results indicated that the protective and anti-inflammatory effects of CGA on human SW-1353 chondrocytes may occur via the NF- κ B pathway.

In conclusion, the present study demonstrated the *in vitro* anti-inflammatory and cartilage-protective role of CGA. The underlying mechanism appeared to involve the inhibition of COX-2, PGE₂, iNOS, NO, IL-6 and MMP-13. Furthermore, IL-1 β -induced collagen II destruction was abrogated via the inhibition of I κ B α /NF- κ B activation. These results suggested that CGA may be considered a novel therapeutic candidate against arthritic diseases. Future studies should fully clarify the molecular mechanisms underlying the effects of CGA on chondrocytes, as there may be additional signaling pathways involved. *In vivo* investigations of CGA in animals and humans are required to further investigate the potential of CGA in the clinical management of OA.

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