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Isoliquiritigenin Inhibits IL-1β-Induced Production of Matrix Metalloproteinase in Articular Chondrocytes

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Osteoarthritis (OA) is a major joint disease in which inflammatory cytokine interleukin-1β (IL-1β) and matrix metalloproteinases (MMPs) play a pivotal role. Isoliquiritigenin has been reported to have anti-inflammation activity. In this study, the effect of isoliquiritigenin on IL-1β-induced production of matrix metalloproteinase and nuclear factor κB (NF-κB) activation was analyzed. We treated primary cultured articular chondrocytes with isoliquiritigenin and the expressions of MMPs were analyzed on mRNA and protein level. The phosphorylation of IκBa and p65 was analyzed to detect NF-κB activation. We also used *in vivo* model by treating mice with isoliquiritigenin and detecting the level of MMPs. IL-1β induced NF-κB activation and MMP-1, MMP-3, MMP-9, MMP-13, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and ADAMTS-5 production on chondrocytes. A 10-μM isoliquiritigenin treatment significantly inhibited IL-1β-induced NF-κB activation and these MMPs production on chondrocytes. Injecting isoliquiritigenin into rat knee joint also inhibited IL-1β-induced NF-κB activation and MMPs production in articular cartilage. Isoliquiritigenin treatment inhibited IL-1β-induced MMPs production and NF-κB activation both *in vitro* and *in vivo*, suggesting a potential therapeutic role of isoliquiritigenin to treat osteoarthritis.

INTRODUCTION

Osteoarthritis (OA) is the most common degenerative joint disease, affecting more than 25% of the population over 18 years old.[1] OA is a progressive and dynamic process in joint tissues, including cartilage, underlying bone, the entire synovial joint, synovium, and muscle. One of the main symptoms in OA is progressive degeneration of articular cartilage including chondrocyte loss and degradation of the extracellular matrix (ECM). This phenomenon represents deregulation of chondrocyte metabolism due to the actions of inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α), which is responsible for the downregulation of collagen as well as proteoglycan biosynthesis and stimulation of their degradation.

OA affects predominantly articular cartilage, which degrades by gradual loss of its ECM composed mainly of aggrecan and type II collagen. Loss of large proteoglycan aggrecan decreases cartilage compressive stiffness and precedes the damage to collagen fibrillar network, which is responsible for tensile properties of the tissue.[2] The activation of degradative enzymes leads to the loss and degradation of proteoglycan and collagen in articular cartilage and the MMPs play a pivotal role in the destruction of articular cartilage in OA.[3] MMPs can be classified into collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), and stromelysins (MMP-3, -7, -10, and -11).[4] Among these MMPs, the role of MMP-1, -13, -2, -3, and -9 in OA has been reported.[5] In addition, the anti-OA effects of inhibitors targeting these MMP have also been reported,[3,6–11] indicating inhibiting MMP activities should be an effective therapy to treat OA.

Isoliquiritigenin (ISL) is a flavonoid derived from *Glycyrrhiza uralensi*. It has diverse biological activities including anti-allergic,[12] anti-angiogenesis,[13] anti-tumor growth,[14] and anti-inflammation.[15–18] For the anti-inflammatory activity, ISL has been reported to inhibit LPS-induced nuclear factor κB (NF-κB) activation[16] and MAPK activation.[18,19] IL-1β can activate NF-κB and induce MMPs production,[20,21] playing an essential role in OA pathophysiology. Thus, in this study, we tested the potential role of ISL on IL-1β-induced MMPs production and NF-κB activation.

RESULTS

ISL Showed No Effects on Primary Rat Chondrocytes Proliferation (Cytotoxicity Assay)

The primary rat chondrocytes were treated with 5, 10, and 20 μM ISL for 72 hr and then SRB assay was performed to determine the cytotoxicity of ISL. As shown in Figure 1, there was no significant difference of cell viability in cell samples treated with 5, 10, and 20 μM ISL when compared to control. These data indicated that the selected concentration of ISL did not affect the cell viability of primary rat chondrocytes in long-term cultures and we used these concentrations for following experiments.

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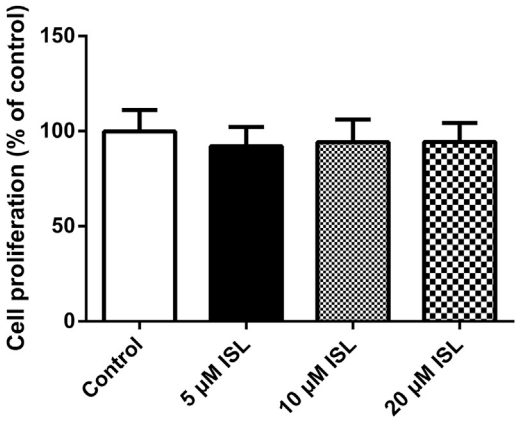

Figure 1. Effect of Isoliquiritigenin on Proliferation of Rabbit Chondrocytes
Chondrocytes were incubated for 72 hr in the presence of varying concentrations of isoliquiritigenin. Cell viability was determined using SRB assay as described in Materials and Methods. Each bar represents a mean ± SEM of three independent experiments in comparison with that of the control set at 100%.

Effects of ISL on IL-1β-Induced Gene Expression of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5

IL-1β has been considered the central mediator of cartilage loss in OA and upregulates the major extracellular proteolytic enzymes in cartilage degradation, such as MMPs and a disintegrin-like and metalloproteinases with thrombospondin motifs (ADAMTS).[5] To test the effects of ISL on the gene expression of MMPs and ADAMTS induced by IL-1β, primary articular chondrocytes were pretreated with varying concentrations of ISL for 2 hr and then stimulated with IL-1β for 24 hr. The gene expressions of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 were analyzed by real-time PCR. The relative mRNA expressions of these genes were shown in Figure 2. IL-1β treatment significantly induced gene expression of MMP-1 while 5 μM ISL treatment significantly inhibited the MMP-1 gene expression induced by IL-1β. The inhibition effects were in a dose-dependent manner as 20 μM exhibited the best inhibition effect (Figure 2A). A 20 μM ISL treatment alone did not affect the gene expression. Similarly, IL-1β treatment also significantly induced gene expression of MMP-3 (Figure 2B), MMP-9 (Figure 2C), MMP-13 (Figure 2D), ADAMTS-4 (Figure 2E) and ADAMTS-5 (Figure 2F). A 5 μM ISL treatment significantly inhibited the MMP-13 gene expression induced by IL-1β while 10 μM ISL treatment significantly inhibited the MMP-3, MMP-9, ADAMTS-4, and ADAMTS-5 gene expression induced by IL-1β. All the inhibition effects were in a dose-dependent manner.

Effects of Isoliquiritigenin on the Protein Level of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5

As we detected the inhibition of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 mRNA expression levels after ISL treatment, we further determined the protein levels by western blot. Consistent to mRNA level, we detected increased protein levels of all six proteins after IL-1β stimulation. Once cells were treated with ISL, we detected decreased protein level in a dose-dependent manner (Figure 3A). The relative protein level was quantitated (Figure 3B) and 10 μM ISL treatment significantly decreased the protein levels of all six proteins after IL-1β stimulation. Thus, our data indicated that ISL regulates IL-1β-induced production of MMP in primary cultured articular chondrocytes.

ISL Inhibited IL-1β-Induced NF-κB Activation

IL-1β can initiate several signal transduction pathways, leading to an increase in intracellular Ca^{2+}, activation of PKC, p38, ERK1/2, and JNK, and nuclear translocation of NF-κB, activating transcription factor (ATF), and activator protein 1 (AP1).[20] As ISL had been shown to be able to inhibit the NF-κB activation in several different models, we continued to test whether ISL also inhibited the IL-1β-induced NF-κB activation in primary cultured articular chondrocytes. As shown in Figure 4A, IL-1β treatment activated NF-κB activation as increased phosphorylated-p65 and phosphorylated-IκBα detected in IL-1β alone samples. In the presence of ISL, the phosphorylation of both p65 and IκBα decreased. After quantitation, we detected significantly decreased protein level of p-p65 and p-IκBα in the presence of 5-μM ISL (Figure 4B), indicating ISL inhibited IL-1β-induced NF-κB activation, which was in a dose-dependent manner.

ISL Inhibited IL-1β-Induced Production of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, ADAMTS-5, and NF-κB Activation *In Vivo*

To investigate whether ISL also shows the potential effect *in vivo*, we examined the effect of intra-articular injection of ISL into the knee joint of rats on IL-1β-stimulated production of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 as well as NF-κB activation from articular cartilage tissues. As shown in Figures 5A and 5C, treatment with IL-1β (20 ng/20 μL) activated NF-κB and increased MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 production in articular cartilage tissues. The treatment of ISL inhibited NF-κB activation and decreased protein levels of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5. After quantitation, treatment of 20 mg/kg ISL significantly inhibited IL-1β-induced NF-κB activation and suppressed MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 production (Figures 5B and 5D). The inhibition effects were in a dose-dependent manner; treatment of 100 mg/kg ISL showed the best inhibition effects. Thus, our data indicated that ISL also functioned *in vivo*.

DISCUSSION

OA, a common form of arthritis, is an age-related degenerative disease characterized by the chronic joint pain, inflammation and the damage of joint cartilage. The degeneration is mainly characterized by a progressive degradation of extracellular matrix (ECM) components, followed by chondrocyte death, tissue fibrillation, and erosion.[22]

OA affects predominantly articular cartilage, which degrades by gradual loss of its ECM composed mainly of aggrecan and type II

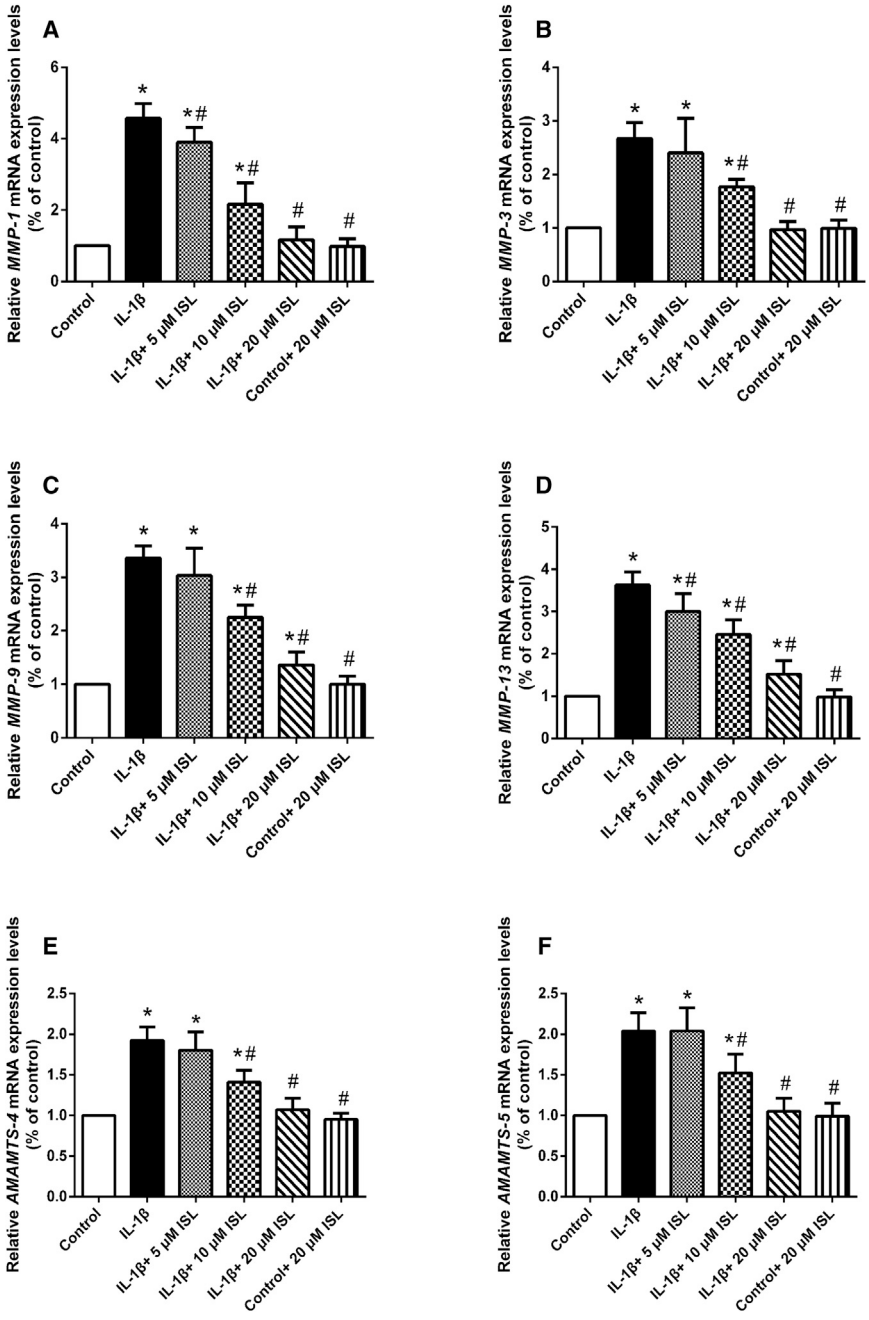

Figure 2. Effect of Isoliquiritigenin on the Gene Expression of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 in Chondrocytes

Primary cultured articular chondrocytes were pretreated with varying concentrations of isoliquiritigenin for 2 hr and then stimulated with IL-1β (10 ng/mL) for 24 hr. The gene expression levels of MMP-1 (A), MMP-3 (B), MMP-9 (C), MMP-13 (D), ADAMTS-4 (E), and ADAMTS-5 (F) were measured by real-time PCR. Three independent experiments were performed and the representative data were shown. Each bar represents a mean ± SEM of three independent experiments in comparison with that of the control set at 100%. *Significantly different from control ($p < 0.05$). Significantly different from IL-1β alone (#$p < 0.05$).

collagen. Loss of large proteoglycan aggrecan decreases cartilage compressive stiffness and precedes the damage to collagen fibrillar network, which is responsible for tensile properties of the tissue. Aggrecan degradation is associated with upregulation of aggrecanases ADAMTS-4 and-5 as well as MMPs.[23] The excessive cleavage of type II collagen in OA is assumed to be caused by the upregulation of the synthesis and activities of collagenases,[24,25] in particular MMP-13.[26,27] Presently, it is believed that articular cartilage destruction in OA results from excessive loading, age-related changes, and metabolic imbalance in the tissue.[28]

OA also exhibits features of a systemic disease as it has been shown to involve vascular pathology as well as T cell immune response associated with upregulation of cytokines such as IL-1β and TNF-α,[29] which aggravate cartilage resorption. IL-1β has been reported to induce MMP-1, MMP-3, MMP-13 and ADAMTS-4 in human tendon cells[20] and promote MMP-1 and MMP-2 expression in human aortic valve myofibroblasts.[30] In this study, we also detected that IL-1β activated NF-κB and induced production of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 in articular chondrocytes both *in vivo* and *in vitro* (Figures 2, 3, and 5), suggesting the essential role of IL-1β and MMPs in OA.

As the mechanism of OA development is not completely understood, the disease manifestations, which are associated with cartilage resorption and inflammation, suggest a treatment involving inhibition of proinflammatory cytokines or MMP activity to prevent matrix destruction.[2] IL-1β activity is mediated solely by binding to its specific receptor, IL-1RI and induces phosphorylation of dependent signaling pathways with p38 MAP kinase and NF-κB as principal pathways that regulate various gene expression, including the synthesis of several inflammatory cytokines and MMPs.[5] Specific inhibitions of IL-1β through the application of IL-1 receptor antagonist protein, soluble IL-1 receptors, monoclonal antibodies against IL-1β, blocking the formation of active IL-1β, blocking the IL-1β cellular signaling pathways have been used as treatment to OA.[31] Using MMP-13 inhibitor CL82198, Wang and colleagues found CL82198 inhibited MMP-13 activity in media from primary murine chondrocytes and intraperitoneal injection of CL82198 decelerated OA progression,

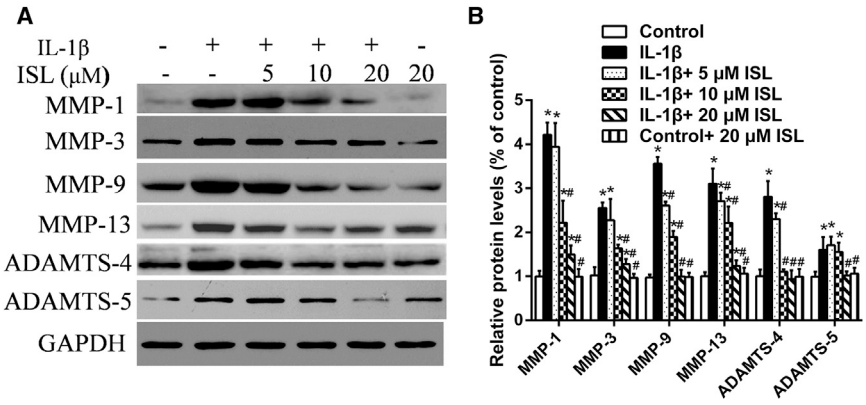

Figure 3. Effect of Isoliquiritigenin on the Protein Levels of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 in Chondrocytes

Primary cultured articular chondrocytes were pretreated with varying concentrations of isoliquiritigenin for 2 hr and then stimulated with IL-1β (10 ng/mL) for 24 hr. The protein levels of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 were measured by western blotting (A). Relative protein expressions to control were shown as histogram in (B). Six independent experiments were performed and the representative data were shown. Each bar represents a mean ± SEM of three independent experiments in comparison with that of the control set at 100%. Significantly different from control (*$p < 0.05$). Significantly different from IL-1β alone (#$p < 0.05$).

increased type II collagen and proteoglycan levels.[7] Baragi et al.[9] found ALS 1-0635, a MMP-13 inhibitor, inhibited bovine articular cartilage degradation in a dose-dependent manner and modulated cartilage damage in rat model. Thus, the inhibitors exert chondroprotective effects and can potentially modulate joint pain, and are, therefore, uniquely suited as potential disease-modifying OA drugs. However, these treatments were not entirely satisfactory, and searching for new drugs is required to achieve the desired goals of therapy.

ISL is a flavonoid derived from licorice compounds and showed various biological activities including antioxidant and anti-inflammatory properties.[32,33] A previous study has revealed ISL could significantly inhibit cytokine-induced endothelial cell adhesion molecule expression through NF-κB. ISL could also dampen MMP-1 and MMP-2 production via inhibition of MAPK-responsive signaling pathway.[34] These previous studies strongly suggested ISL, which can inhibit MMPs production and NF-κB activation, could have the potential to treat OA. Thus, we tested the possible inhibitory effect of ISL on MMPs production and NFκB activation in our *in vitro* primary cultured articular chondrocytes model. We found that ISL is quite safe as the highest concentration of 20 μM did not cause toxicity on chondrocytes (Figure 1) while 5 μM ISL treatment significantly prevented IL-1β-induced MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 production (Figures 2 and 3) and NF-κB activation (Figure 4). The inhibition effect of ISL on MMPs production was reflected on both mRNA protein level, suggesting the ISL inhibited the transcriptions of MMPs. As we detected the inhibition of NF-κB at the same time, it is clear that the inhibition of MMPs production by ISL was through NF-κB pathway. Furthermore, we found the inhibitory effect of ISL on IL-1β-induced MMPs production and NF-κB activation also existed *in vivo*. We detected decreased protein levels of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 from rat articular cartilage lysate, together with decreased NF-κB activation (Figure 5). Taking together, our data showed ISL exerts chondroprotective effects both *in vivo* and *in vitro*, suggesting that ISL as a novel agent for the control of cartilage damage in OA.

In summary, our experiments showed that the chondroprotective effects of ISL are produced by inhibiting IL-1β-induced production of MMPs through NF-κB.

MATERIALS AND METHODS

Primary Cultures of Chondrocytes from Rat Articular Cartilage

A modified method for harvesting chondrocytes was performed as previously described.[35] Briefly, rat chondrocytes were isolated from articular cartilage in 3-week-old male Sprague Dawley rats. Cartilage was removed from animals that were subsequently

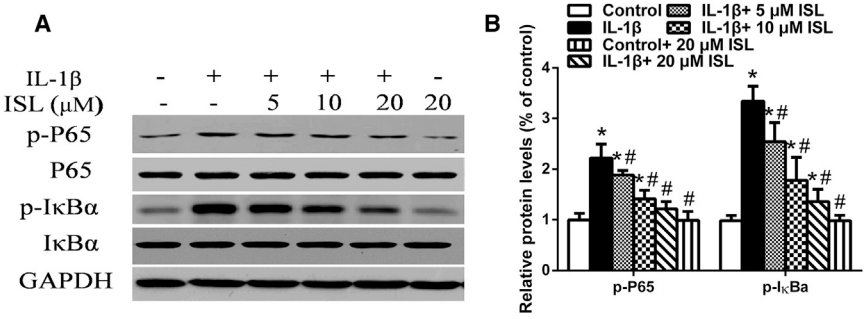

Figure 4. Effect of Isoliquiritigenin on Phosphorylation of NF-κB and IκBα in Chondrocytes

Primary cultured articular chondrocytes were pretreated with varying concentrations of isoliquiritigenin for 2 hr and then stimulated with IL-1β (10 ng/mL) for 24 hr. The phosphorylation of NF-κB and IκBα were measured by western blotting (A). Relative protein expressions to control were shown as histogram in (B). Six independent experiments were performed and the representative data were shown. Each bar represents a mean ± SEM of three independent experiments in comparison with that of the control set at 100%. Significantly different from control (*$p < 0.05$). Significantly different from IL-1β alone (#$p < 0.05$).

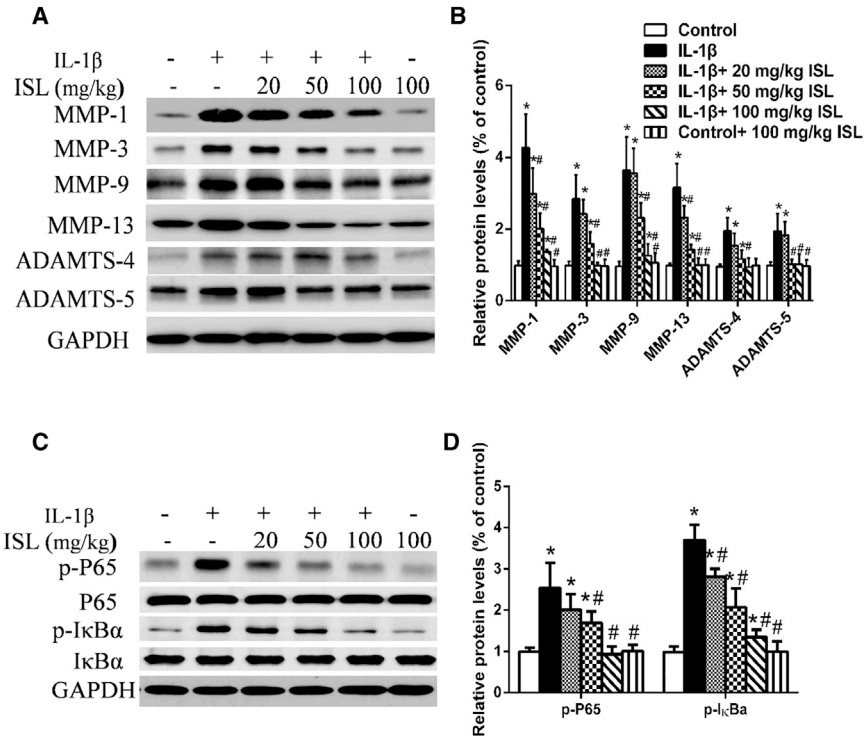

Figure 5. Effect of Isoliquiritigenin on the Protein Levels of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, ADAMTS-5 and the Phosphorylation of NF-κB and IκBα *In Vivo*

The knee joint of rats was pretreated with 20, 50, or 100 mg/kg of isoliquiritigenin for 3 hr and then stimulated with IL-1β (20 ng/20 μL) for 72 hr by intra-articular injection. Tissue lysates from articular cartilage homogenates were collected for measuring the protein levels of MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5 (A) and the phosphorylation of NF-κB and IκBα (C) *in vivo* by western blot analysis. Relative protein expressions to control were shown in (B) and (D), respectively. Six independent experiments were performed and the representative data were shown. Each bar represents a mean ± SEM of three independent experiments in comparison with that of the control set at 100%. Significantly different from control (*$p < 0.05$). Significantly different from IL-1β alone (#$p < 0.05$).

euthanized by an overdose of anesthesia. The cartilage was cut into thin slices and then washed with sterilized PBS and soaked in 5% penicillin-streptomycin-neomycin (Sigma, St. Louis, MO, USA) for 15 min. The cartilage slices were washed with PBS to remove residual antibiotic solution and digested with 0.02% collagenase type II (Sigma, USA) in DMEM (HyClone, Logan, UT, USA) for 3 hr in a 37°C water bath. Digested cartilage was collected and centrifuged. The pellet was resuspended in DMEM and filtered through 70 μm nylon mesh. The resultant rat chondrocytes were cultured in DMEM supplement with 10% fetal bovine serum and 1% penicillin-streptomycin-neomycin in a 5% CO_2 incubator at 37°C.

Treatment of Cells with ISL

Chondrocytes were seeded on 6-well culture plates with a density of 2×10^5 cells/mL. After 2 days in monolayer culture, the cells were incubated for 2 hr in growth medium with 5, 10, or 20 μM ISL, followed by incubation in the presence or absence of 10 ng/mL IL-1β for 24 hr. ISL was purchased from Shaanxi Green Bio-Engineering (Xi'an, China) and dissolved in 1% sodium carboxymethyl cellulose (CMC-Na).

Cytotoxicity Assay

Chondrocytes were seeded at a density of 2×10^5/mL (0.1 mL/well) in a 96-well microtiter plate and allowed to attach for 24 hr to keep the log phase growth at the time of drug treatment. After incubation with the indicated drug concentrations for 72 hr, cell proliferation was determined using the sulforhodamine B (SRB) assay (Sigma, St. Louis, MO, USA).

Quantitative Real-Time PCR

RNA isolation was performed by using the RNeasy mini kit (QIAGEN, Valencia, CA, USA). The Quatitect reverse transcription kit (QIAGEN, USA) was used to synthesize the subsequent cDNA. Real-time PCR was performed using SYBR Green Master Mix (QIAGEN, USA). Samples were normalized to internal control GAPDH. Primer sequences used for real-time PCR are listed in Table 1.

In Vivo Experiments

Male Sprague-Dawley rats weighing 200–210 g were used to investigate the effect of ISL in articular cartilage *in vivo*. Animals were housed five per cage, provided with distilled water and food *ad libitum*, and kept under a 12 hr light/dark cycle at constant temperature (22.5°C) and humidity (55%). Animals were cared for by the Care and Use of Laboratory Animals of the National Institutes of Health of Cangzhou Central Hospital. The rats were randomly divided into six groups: control, IL-1β only, 20 mg/kg ISL plus IL-1β, 50 mg/kg ISL plus IL-1β, 100 mg/kg ISL plus IL-1β or 100 mg/kg ISL alone. Rats were anesthetized with vaporized diethylether, and those from the ISL plus IL-1β or ISL alone groups received a 30 μL injection of different amounts of ISL, respectively, into the right knee joint. After 3 hr, rats received a 30 μL injection of 20 ng IL-1β in sterile PBS into the right knee joint. Rats from the control group were injected with 30 μL of sterile PBS. Rats were euthanized via CO_2 asphyxiation 72 hr after injections. Articular cartilage (tibial plateau and femoral condyle) was isolated from each animal, homogenized, and prepared for measurement of protein by western blot analysis.

Western Blot

A total of 50 μg of proteins from either cell lysate or articular cartilage homogenate were loaded onto a 12% SDS-PAGE gel. After transfer, membranes were blocked by 5% non-fat milk and incubated with

Table 1. Primer Sequences Used for Real-Time PCR

Gene	Primer	Sequences (5′-3′)
MMP-1	forward	CCGGCAGAATGTGGAAACAG
	reverse	GCTGCATTTGCCTCAGCTTT
MMP-3	forward	TTTGGCCGTCTCTTCCATCC
	reverse	GGAGGCCCAGAGTGTGAATG
MMP-9	forward	AGGGCCCCTTTCTTATTGCC
	reverse	CGAGTAACGCTCTGGGGATC
MMP-13	forward	GGACTCACTGTTGGTCCCTG
	reverse	GGATTCCCGCAAGAGTCACA
ADAMTS-4	forward	CATCCTACGCCGGAAGAGTC
	reverse	AAGCGAAGCGCTTGTTTCTG
ADAMTS-5	forward	CCCAAATACGCAGGTGTCCT
	reverse	ACACACGGAGTTGCTGTAGG
Gapdh	forward	TGTGAACGGATTTGGCCGTA
	reverse	TGAACTTGCCGTGGGTAGAG

different primary antibodies for overnight at 4°C. Next day, corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies were incubated. After washing, the membranes were finally carried out with SuperSignal1 West Pico Chemiluminescent Substrate Kit (Thermo Scientific, Waltham, MA, USA). Rabbit anti-MMP1 (1:2,000), rabbit anti-MMP3 (1:2,000), rabbit anti-MMP9 (1:2,000), rabbit anti-MMP13 (1:2,000), rabbit anti-ADAMTS4 (1:2,000), rabbit anti-ADAMTS5 (1:2,000), mouse anti-GAPDH (1:1,000), rabbit anti-IκBa (1:2,000), rabbit anti-p- IκBa (1:2,000), rabbit anti-p65 (1:2,000), rabbit anti-p-p65 (1:2,000), goat anti-mouse immunoglobulin G H&L (HRP) (1:3,000), and goat anti-rabbit immunoglobulin G (HRP) (1:3,000) were purchased from Abcam (Cambridge, MA, USA). The western blot results were quantitated and analyzed using GS-900 Calibrated Densitometer and software Image Lab (Bio-Rad, Hercules, CA, USA) following manufacturer's instructions.

Statistical Analysis

One-way ANOVA analysis, followed by a Tukey's post hoc test was used to determine the related protein levels. Statistical difference was considered as significant only if $p < 0.05$.

AUTHOR CONTRIBUTIONS

Designed the study, did the experiments, and wrote the manuscript: L.Z.; did the experiments and analyzed the data: S.M., H.S., and J.C.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Chen, D., Shen, J., Zhao, W., Wang, T., Han, L., Hamilton, J.L., and Im, H.J. (2017). Osteoarthritis: toward a comprehensive understanding of pathological mechanism. Bone Res. *5*, 16044.
2. Tchetina, E.V. (2011). Developmental mechanisms in articular cartilage degradation in osteoarthritis. Arthritis (Egypt) *2011*, 683970.
3. Park, J.S., Kim, D.K., Shin, H.D., Lee, H.J., Jo, H.S., Jeong, J.H., Choi, Y.L., Lee, C.J., and Hwang, S.C. (2016). Apigenin Regulates Interleukin-1β-Induced Production of Matrix Metalloproteinase Both in the Knee Joint of Rat and in Primary Cultured Articular Chondrocytes. Biomol. Ther. (Seoul) *24*, 163–170.
4. Visse, R., and Nagase, H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ. Res. *92*, 827–839.
5. Burrage, P.S., Mix, K.S., and Brinckerhoff, C.E. (2006). Matrix metalloproteinases: role in arthritis. Front. Biosci. *11*, 529–543.
6. Xie, X.W., Wan, R.Z., and Liu, Z.P. (2017). Recent Research Advances in Selective Matrix Metalloproteinase-13 Inhibitors as Anti-Osteoarthritis Agents. ChemMedChem *12*, 1157–1168.
7. Wang, M., Sampson, E.R., Jin, H., Li, J., Ke, Q.H., Im, H.J., and Chen, D. (2013). MMP13 is a critical target gene during the progression of osteoarthritis. Arthritis Res. Ther. *15*, R5.
8. Ni, G.X., Zhan, L.Q., Gao, M.Q., Lei, L., Zhou, Y.Z., and Pan, Y.X. (2011). Matrix metalloproteinase-3 inhibitor retards treadmill running-induced cartilage degradation in rats. Arthritis Res. Ther. *13*, R192.
9. Baragi, V.M., Becher, G., Bendele, A.M., Biesinger, R., Bluhm, H., Boer, J., Deng, H., Dodd, R., Essers, M., Feuerstein, T., et al. (2009). A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: Evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models. Arthritis Rheum. *60*, 2008–2018.
10. Yang, C.C., Lin, C.Y., Wang, H.S., and Lyu, S.R. (2013). Matrix metalloproteases and tissue inhibitors of metalloproteinases in medial plica and pannus-like tissue contribute to knee osteoarthritis progression. PLoS ONE *8*, e79662.
11. Sabatini, M., Lesur, C., Thomas, M., Chomel, A., Anract, P., de Nanteuil, G., and Pastoureau, P. (2005). Effect of inhibition of matrix metalloproteinases on cartilage loss in vitro and in a guinea pig model of osteoarthritis. Arthritis Rheum. *52*, 171–180.
12. Kakegawa, H., Matsumoto, H., and Satoh, T. (1992). Inhibitory effects of some natural products on the activation of hyaluronidase and their anti-allergic actions. Chem. Pharm. Bull. (Tokyo) *40*, 1439–1442.
13. Wang, Z., Wang, N., Han, S., Wang, D., Mo, S., Yu, L., Huang, H., Tsui, K., Shen, J., and Chen, J. (2013). Dietary compound isoliquiritigenin inhibits breast cancer neoangiogenesis via VEGF/VEGFR-2 signaling pathway. PLoS ONE *8*, e68566.
14. Lee, C.K., Son, S.H., Park, K.K., Park, J.H., Lim, S.S., and Chung, W.Y. (2008). Isoliquiritigenin inhibits tumor growth and protects the kidney and liver against chemotherapy-induced toxicity in a mouse xenograft model of colon carcinoma. J. Pharmacol. Sci. *106*, 444–451.
15. Du, F., Gesang, Q., Cao, J., Qian, M., Ma, L., Wu, D., and Yu, H. (2016). Isoliquiritigenin Attenuates Atherogenesis in Apolipoprotein E-Deficient Mice. Int. J. Mol. Sci. *17*, 17.
16. Kim, J.Y., Park, S.J., Yun, K.J., Cho, Y.W., Park, H.J., and Lee, K.T. (2008). Isoliquiritigenin isolated from the roots of Glycyrrhiza uralensis inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kappaB in RAW 264.7 macrophages. Eur. J. Pharmacol. *584*, 175–184.
17. Kumar, S., Sharma, A., Madan, B., Singhal, V., and Ghosh, B. (2007). Isoliquiritigenin inhibits IkappaB kinase activity and ROS generation to block TNF-alpha induced expression of cell adhesion molecules on human endothelial cells. Biochem. Pharmacol. *73*, 1602–1612.
18. Choi, Y.H., Bae, J.K., Chae, H.S., Choi, Y.O., Nhoek, P., Choi, J.S., and Chin, Y.W. (2016). Isoliquiritigenin ameliorates dextran sulfate sodium-induced colitis through the inhibition of MAPK pathway. Int. Immunopharmacol. *31*, 223–232.
19. Wu, S., Xue, J., Yang, Y., Zhu, H., Chen, F., Wang, J., Lou, G., Liu, Y., Shi, Y., Yu, Y., et al. (2015). Isoliquiritigenin Inhibits Interferon-γ-Inducible Genes Expression in Hepatocytes through Down-Regulating Activation of JAK1/STAT1, IRF3/MyD88, ERK/MAPK, JNK/MAPK and PI3K/Akt Signaling Pathways. Cell. Physiol. Biochem. *37*, 501–514.
20. Tsuzaki, M., Guyton, G., Garrett, W., Archambault, J.M., Herzog, W., Almekinders, L., Bynum, D., Yang, X., and Banes, A.J. (2003). IL-1 beta induces COX2, MMP-1, -3 and -13, ADAMTS-4, IL-1 beta and IL-6 in human tendon cells. J. Orthop. Res. *21*, 256–264.

21. Kapoor, M., Martel-Pelletier, J., Lajeunesse, D., Pelletier, J.P., and Fahmi, H. (2011). Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat. Rev. Rheumatol. *7*, 33–42.

22. Martel-Pelletier, J. (1999). Pathophysiology of osteoarthritis. Osteoarthritis Cartilage *7*, 371–373.

23. Nagase, H., and Kashiwagi, M. (2003). Aggrecanases and cartilage matrix degradation. Arthritis Res. Ther. *5*, 94–103.

24. Poole, A.R., Nelson, F., Dahlberg, L., Tchetina, E., Kobayashi, M., Yasuda, T., Laverty, S., Squires, G., Kojima, T., Wu, W., and Billinghurst, R.C. (2003). Proteolysis of the collagen fibril in osteoarthritis. Biochem. Soc. Symp. (70), 115–123.

25. Poole, A.R., Kobayashi, M., Yasuda, T., Laverty, S., Mwale, F., Kojima, T., Sakai, T., Wahl, C., El-Maadawy, S., Webb, G., et al. (2002). Type II collagen degradation and its regulation in articular cartilage in osteoarthritis. Ann. Rheum. Dis. *61* (*Suppl 2*), ii78–ii81.

26. Billinghurst, R.C., Dahlberg, L., Ionescu, M., Reiner, A., Bourne, R., Rorabeck, C., Mitchell, P., Hambor, J., Diekmann, O., Tschesche, H., et al. (1997). Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J. Clin. Invest. *99*, 1534–1545.

27. Dahlberg, L., Billinghurst, R.C., Manner, P., Nelson, F., Webb, G., Ionescu, M., Reiner, A., Tanzer, M., Zukor, D., Chen, J., et al. (2000). Selective enhancement of collagenase-mediated cleavage of resident type II collagen in cultured osteoarthritic cartilage and arrest with a synthetic inhibitor that spares collagenase 1 (matrix metalloproteinase 1). Arthritis Rheum. *43*, 673–682.

28. Aigner, T., Haag, J., Martin, J., and Buckwalter, J. (2007). Osteoarthritis: aging of matrix and cells–going for a remedy. Curr. Drug Targets *8*, 325–331.

29. Malemud, C.J. (2010). Anticytokine therapy for osteoarthritis: evidence to date. Drugs Aging *27*, 95–115.

30. Kaden, J.J., Dempfle, C.E., Grobholz, R., Tran, H.T., Kiliç, R., Sarikoç, A., Brueckmann, M., Vahl, C., Hagl, S., Haase, K.K., and Borggrefe, M. (2003). Interleukin-1 beta promotes matrix metalloproteinase expression and cell proliferation in calcific aortic valve stenosis. Atherosclerosis *170*, 205–211.

31. Jotanovic, Z., Mihelic, R., Sestan, B., and Dembic, Z. (2012). Role of interleukin-1 inhibitors in osteoarthritis: an evidence-based review. Drugs Aging *29*, 343–358.

32. Zhang, X., Yeung, E.D., Wang, J., Panzhinskiy, E.E., Tong, C., Li, W., and Li, J. (2010). Isoliquiritigenin, a natural anti-oxidant, selectively inhibits the proliferation of prostate cancer cells. Clin. Exp. Pharmacol. Physiol. *37*, 841–847.

33. Traboulsi, H., Cloutier, A., Boyapelly, K., Bonin, M.A., Marsault, É., Cantin, A.M., and Richter, M.V. (2015). The Flavonoid Isoliquiritigenin Reduces Lung Inflammation and Mouse Morbidity during Influenza Virus Infection. Antimicrob. Agents Chemother. *59*, 6317–6327.

34. Kang, S.W., Choi, J.S., Choi, Y.J., Bae, J.Y., Li, J., Kim, D.S., Kim, J.L., Shin, S.Y., Lee, Y.J., Kwun, I.S., and Kang, Y.H. (2010). Licorice isoliquiritigenin dampens angiogenic activity via inhibition of MAPK-responsive signaling pathways leading to induction of matrix metalloproteinases. J. Nutr. Biochem. *21*, 55–65.

35. Goldring, M.B. (2006). Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. Best Pract. Res. Clin. Rheumatol. *20*, 1003–1025.