*Inflammation, Vol. 42, No. 6, December 2019 (© 2019)*
DOI: 10.1007/s10753-019-01092-0

ORIGINAL ARTICLE

# Isorhapontigenin Suppresses Interleukin-1β-Induced Inflammation and Cartilage Matrix Damage in Rat Chondrocytes

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*Abstract*— Osteoarthritis (OA) is a common cause of joint pain and physical disability in the elderly. It is highly associated with local inflammatory reactions and cartilage degradation. Isorhapontigenin (ISO), a natural compound existing in various plants, has shown prominent anti-inflammatory and anti-oxidative properties in several inflammatory diseases. However, the effects of ISO on OA remain to be elucidated. Here, we investigated the effects of ISO on interleukin-1β (IL-1β)-treated rat chondrocytes and cartilage explants. Our results revealed that ISO could suppress the IL-1β-induced elevated levels of nitric oxide (NO), inducible nitric oxide synthase (iNOS), prostaglandin E2 (PGE2), and cyclooxygenase-2 (COX2). Besides, ISO could also inhibit the IL-1β-induced up-regulation of cartilage matrix catabolic enzymes such as matrix metalloproteinases (MMPs) and aggrecanase-2 (ADAMTS5). Moreover, the IL-1β-induced downregulation of collagen II and aggrecan could be reversed by ISO. Furthermore, ISO prevented rat cartilage explant damage induced by IL-1β. Mechanistically, ISO worked partly by suppressing mitogen-activated protein kinase (MAPK)-associated ERK and p38 pathways. Taken together, our study indicated the anti-inflammatory potential of ISO on IL-1β-treated rat chondrocytes, providing a new idea for OA treatment.

**KEY WORDS:** osteoarthritis; isorhapontigenin; iNOS; COX2; MMPs; ADAMTS5.

## INTRODUCTION

Osteoarthritis (OA), characterized by synovium inflammation and cartilage degeneration, is the most common chronic progressive joint disease [1]. Clinical features of OA included joint swelling, pain, subchondral bone sclerosis, and stiffness [2]. To date, medical strategies targeting OA are mainly based on pain alleviation and function restoration [3]. Due to shortage of effective measures to delay OA progression, end stage of OA handling is restricted to joint replacement surgery [4].

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Local inflammatory response has been considered as critical factor contributing to the OA development [5]. High level of inflammatory cytokine interleukin-1β (IL-1β) was detected in the cartilage and synovial tissues of OA patients [6]. A previous study indicated that IL-1β could dramatically promote the synthesis of cartilage matrix catabolic enzymes including matrix metalloproteinases (MMPs) and aggrecanase-2 (ADAMTS5) [7]. Additionally, IL-1β had been proven to induce the degradation of the major extracellular matrix components collagen II and aggrecan in chondrocytes [8]. Moreover, pro-inflammatory factors such as cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) could be up-regulated by IL-1β in chondrocytes, which is directly related to the excessive secretion of prostaglandin E2 (PGE2) and nitric oxide (NO) [9]. Strategies antagonizing IL-1β-induced inflammation may provide new ways for OA therapy.

Isorhapontigenin (ISO), a natural derivative of stilbene, has been widely found in various plants [10]. Previous studies confirmed the prominent anti-inflammatory and anti-oxidative potential of ISO in several diseases [11, 12]. Besides, ISO had been proven to suppress the oxLDL-induced mitogenesis *via* blocking ROS generation and ERK pathway activation in bovine aortic smooth muscle cells, indicating the possible mechanism of its pharmacological action [13]. However, the effects of ISO on OA remain unclear. Therefore, the anti-inflammatory properties as well as the possible underlying mechanism of ISO on IL-1β-treated rat chondrocytes were investigated in this study. We aimed to explore new agents for OA therapy.

## MATERIALS AND METHODS

### Chemicals and Regents

Isorhapontigenin (ISO) was obtained from Sigma Aldrich (St. Louis, MO, USA). ISO was diluted in DMSO for storing and all experiment groups were controlled with equal DMSO concentration. Recombinant rat IL-1β and $PGE_2$ enzyme-linked immunosorbent assay (ELISA) kit were provided by R&D systems (Minneapolis, MN, USA). Fetal bovine serum (FBS) was acquired from Gibco (NY, USA). Antibodies against MMP1 and collagen II were purchased from Proteintech Group (Wuhan, China). Antibodies specific for COX2, MMP3, p-ERK, p-p38, p-JNK, ERK1/2, p38, and JNK were procured from Cell Signaling Technology (Beverly, MA, USA). Antibodies against MMP13 and iNOS were purchased from Abcam (Cambridge, MA, USA). Antibodies specific for GAPDH, ADAMTS5, and corresponding secondary antibodies were obtained from Boster (Wuhan, China). Antibodies specific for aggrecan was purchased from Santa Cruz (CA, USA).

### Cell Culture

All animal experiments were conducted strictly in accordance with the guidelines of Institutional Animal Care and Use Committee, Tongji Medical College, Huazhong University of Science and Technology and approved by the committee. Chondrocytes were harvested by digesting the knee joints of 1-week-old Sprague–Dawley (SD) rats as described previously [14]. Concisely, articular cartilage was firstly cut into small pieces and digested with 0.25% trypsin for 30 min. Afterward, the pieces were fully digested using 0.25% collagenase II at 37 °C for 8 h. Then the cell suspension was centrifuged (1500 rpm) for 5 min to collect primary chondrocytes. The cells were finally cultured in growth medium (GM) including Dulbecco's modified Eagle's medium F12 (DMEM/F12) supplemented with 10% FBS and 1% penicillin/streptomycin cocktail at 37 °C with 5% $CO_2$. Chondrocytes of third passages were used in our study.

### Cell Viability

CCK8 assay (Boster, Wuhan, China) was used to determine the cell viability. Chondrocytes were placed in a 96-well plate at a density of $1 \times 10^4$ cells/well. After adhesion, the cells were treated with various concentrations of ISO in the presence or absence of IL-1β (10 ng/ml) for 24 h. Then each well was added with 100 μl of culture medium containing 10 μl CCK-8 solution and incubated at 37 °C for 1 h. The absorbance of each well was measured with a microplate reader (Bio-Rad, CA, USA).

### NO and PGE2 Measurement

Griess reaction and $PGE_2$ ELISA kit were employed to measure the NO and PGE2 levels, respectively. Chondrocytes were treated with IL-1β (10 ng/ml) either alone or in combination with two concentrations of ISO (10 or 20 μM) for 24 h. The cell culture supernatants were collected and then used for NO and PGE2 detection according to the manufacturer's protocol.

### Western Blotting

Rat chondrocytes were lysed with cold RIPA containing 1% phenylmethylsulfonyl fluoride and phosphatase inhibitor cocktail (Boster, Wuhan, China). Equal amounts of protein samples (25 μg) were separated on 10% sodium

dodecyl sulfate-polyacrylamide gels and transferred to PVDF membranes. The membranes were blocked with 5% bovine serum albumin for 1 h and then incubated with proper primary antibodies at 4 °C overnight. Subsequently, membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. Finally, blots were visualized with enhanced ECL Substrate Kit (Thermo, USA). Relative protein expression was quantified using Image-J software and compared to internal control GAPDH.

### *Ex Vivo* Evaluation by Organ Culture of Cartilage Explants

Articular cartilage explants were collected from the knee joints of 3-week-old SD rats as described before [15]. Concisely, rat knee cartilage was cut into pieces in the same size. The explants were firstly cultured in GM for 2 days and then moved to serum free medium supplemented with 10 ng/ml IL-1β in the presence or absence of 20 μM ISO for further 3 days. Finally, cartilage explants were fixed in 4% paraformaldehyde, paraffin-embedded, sliced coronally, and stained with hematoxylin and eosin (HE). Representative images of each group were presented and groups were quantified according to the extent of cartilage surface disorganization. Grading system used in the explants was based on the surface disorganization area: "0" (absent), "1" (<25%), "2" (25–50%), "3" (50–75%), "4" (>75%).

### Statistical Analysis

Data were shown as mean ± standard deviation (SD). Statistical comparisons were evaluated by one-way ANOVA followed by Tukey *post hoc* test. $P$ <0.05 was considered as statistical significance. All experiments were conducted at least three times.

## RESULTS

### Effects of ISO on Chondrocyte Viability

CCK-8 assay was used to verify the cytotoxic effects of ISO on chondrocytes. As exhibited in Fig. 1, ISO at the concentrations of 5, 10, and 20 μM showed no side effects on rat chondrocyte viability at 24 h either alone or with IL-1β (10 ng/ml). Therefore, ISO with concentrations of 10 and 20 μM was chosen in the subsequent experiments.

### Effects of ISO on IL-1β-Induced Expression of NO, $PGE_2$, iNOS, and COX2 in Rat Chondrocytes

To confirm the inhibition effects of ISO on the production of NO and $PGE_2$ induced by IL-1β in chondrocytes, Griess regent and ELISA kit were employed to measure the NO and PGE2 levels in the cell culture supernatants, respectively. As shown in Fig. 2a and b, ISO could suppress the IL-1β-induced upregulation of NO and $PGE_2$ in a concentration-dependent manner. Western blotting was further employed to verify the effects of ISO on IL-1β-induced protein expression of iNOS and COX2. As exhibited in Fig. 2c and d, cells were treated with IL-1β (10 ng/ml) either alone or with ISO (10 and 20 μM) for 24 h. ISO could dose-dependently inhibit the elevated levels of iNOS and COX2 induced by IL-1β.

### Effects of ISO on Cartilage Matrix Catabolic Enzymes in IL-1β-Treated Rat Chondrocytes

Cartilage matrix catabolic enzymes including MMPs (MMP1, MMP3, MMP13) and ADAMTS5 play a vital

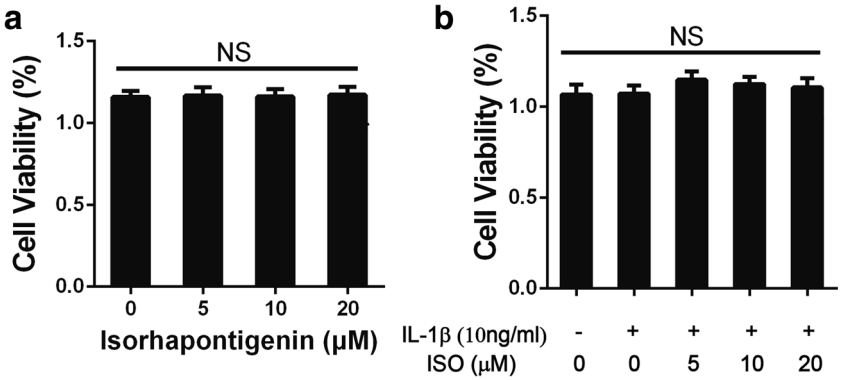


**Fig. 1.** Effects of ISO on rat chondrocyte viability. (**a**) Cells were treated with ISO (5, 10, 20 μM) alone or (**b**) in combination with IL-1β (10 ng/ml) for 24 h, viability was confirmed by CCK-8 assay. NS: no significance.

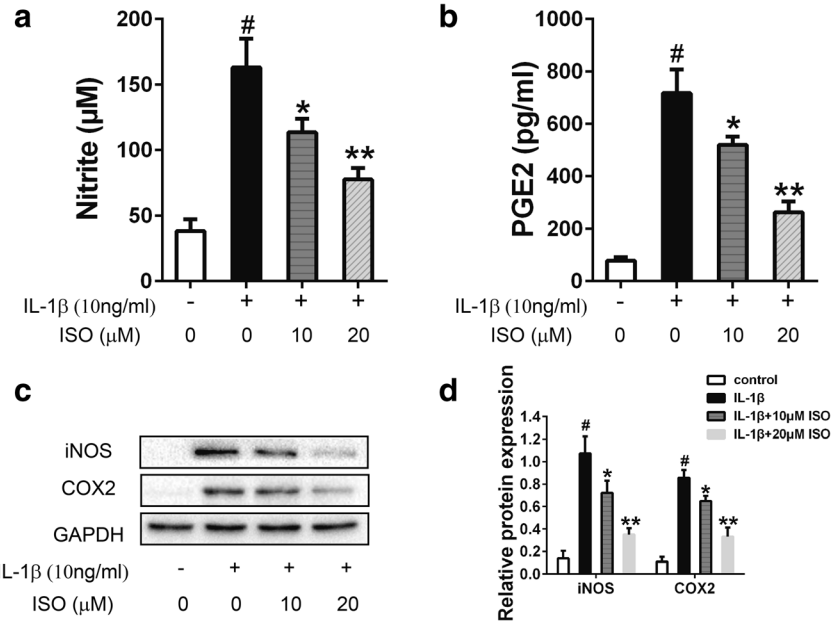


**Fig. 2.** ISO suppressed the expression of NO, $PGE_2$, iNOS, and COX2 in IL-1β-treated rat chondrocytes. Cells were treated with ISO (10, 20 μM) in the absence or presence of IL-1β (10 ng/ml) for 24 h. (**a**) Griess reaction was used to measure the NO levels in the culture supernatants ($n = 3$). (**b**) PGE2 synthesis in the culture supernatants of different groups were evaluated by ELISA ($n = 3$). (**c**) Protein expression of iNOS and COX2 were detected by Western blotting. (**d**) Relative protein expression was quantified using Image-J software, GAPDH was employed as the internal control ($n = 3$). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* IL-1β group; $^{**}P < 0.01$ *vs.* IL-1β group.

role in the cartilage matrix degradation. In this study, cells were treated with IL-1β either alone or in combination with ISO (10 and 20 μM) for 24 h. As presented in Fig. 3, ISO with the concentration of 20 μM could markedly suppress the IL-1β-induced upregulation of MMPs and ADAMTS5.

### Effects of ISO on Extracellular Matrix in IL-1β-Treated Rat Chondrocytes

Collagen II and aggrecan are two key components of cartilage matrix. To explore whether ISO could attenuate IL-1β-induced downregulation of collagen II and aggrecan in rat chondrocytes, western blotting was used for

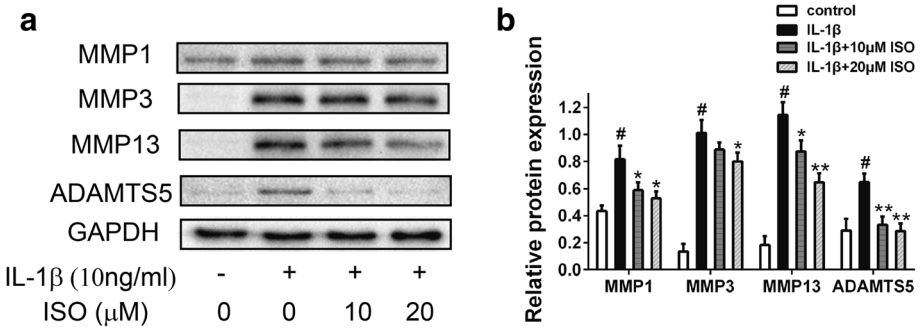


**Fig. 3.** ISO inhibited IL-1β-induced cartilage matrix catabolic enzyme upregulation in rat chondrocytes. Chondrocytes were exposed to ISO (10, 20 μM) alone or in combination with IL-1β (10 ng/ml) for 24 h. (**a**) Protein expression of MMPs and ADAMTS5 were detected by Western blotting. (**b**) Relative protein expression was quantified *via* Image-J software, GAPDH was employed as the loading control ($n = 3$). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* IL-1β group; $^{**}P < 0.01$ *vs.* IL-1β group.

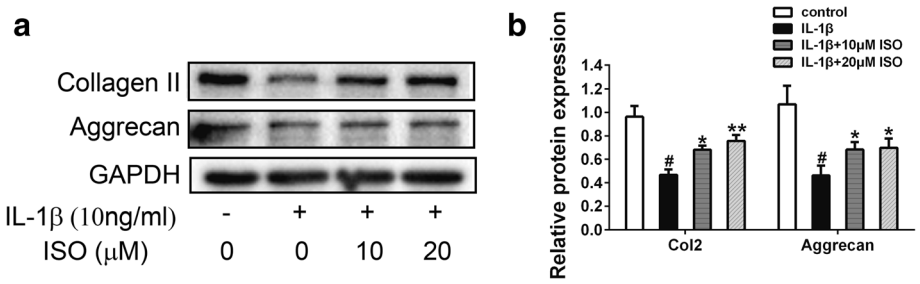


**Fig. 4.** ISO attenuated extracellular matrix degradation in IL-1β-treated rat chondrocytes. Cells were treated exposed to ISO (10, 20 μM) with or without IL-1β (10 ng/ml) for 24 h. (**a**) Protein expression of collagen II and aggrecan were assessed using Western blotting. (**b**) Relative protein expression was quantified with Image-J software, GAPDH was employed as the internal control ($n = 3$). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* IL-1β group; $^{**}P < 0.01$ *vs.* IL-1β group.

evaluation. As shown in Fig. 4, cells were treated with IL-1β in the absence or presence of ISO (10 and 20 μM) for 24 h. Both concentrations of ISO could significantly attenuate the IL-1β-induced degradation of collagen II and aggrecan.

### Effects of ISO on MAPK Pathway Activation in IL-1β-Treated Rat Chondrocytes

MAPK pathway is highly associated with inflammatory activities in OA development. In our study, chondrocytes were firstly serum starved for 12 h and then treated with IL-1β in the absence or presence of ISO (10 and 20 μM) for 30 min. As exhibited in Fig. 5, IL-1β dramatically induced the phosphorylations of ERK, p38, and JNK. However, ISO could block the upregulation of p-ERK and p-p38.

### Effects of ISO on Cartilage Damage in IL-1β-Treated Rat Articular Cartilage Explants

To further confirm the protective effects of ISO on rat OA, an *ex vivo* model by organ culture of rat cartilage explants was built. As shown in Fig. 6, IL-1β notably induced the cartilage surface damage characterized by surface disorganization. In contrast, 20 μM ISO could significantly reverse this change.

## DISCUSSION

Osteoarthritis (OA) is a frequently seen degenerative joint disorder featured with inflammation and cartilage degradation [16]. Current therapies targeting OA work only on the symptoms relief, while effective agents for the OA progression remain to be explored [17]. Due to prominent

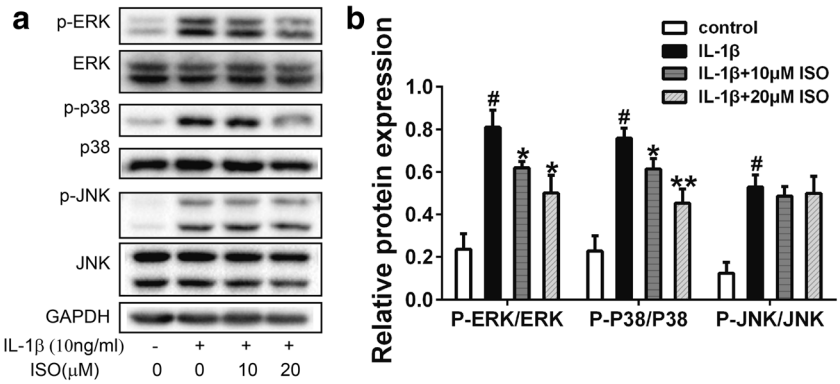


**Fig. 5.** ISO blocked IL-1β-induced MAPK-associated ERK and P38 pathway activation in rat chondrocytes. Chondrocytes were serum starved for 12 h and treated with ISO (10, 20 μM) alone or in combination with IL-1β (10 ng/ml) for 30 min. (**a**) Protein level of p-ERK, p-p38, p-JNK, ERK, p38, and JNK were detected *via* Western blotting. (**b**) Relative protein expression was quantified using Image-J software, total ERK, p38, and JNK were employed as the internal control, respectively ($n = 3$). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* IL-1β group; $^{**}P < 0.01$ *vs.* IL-1β group.

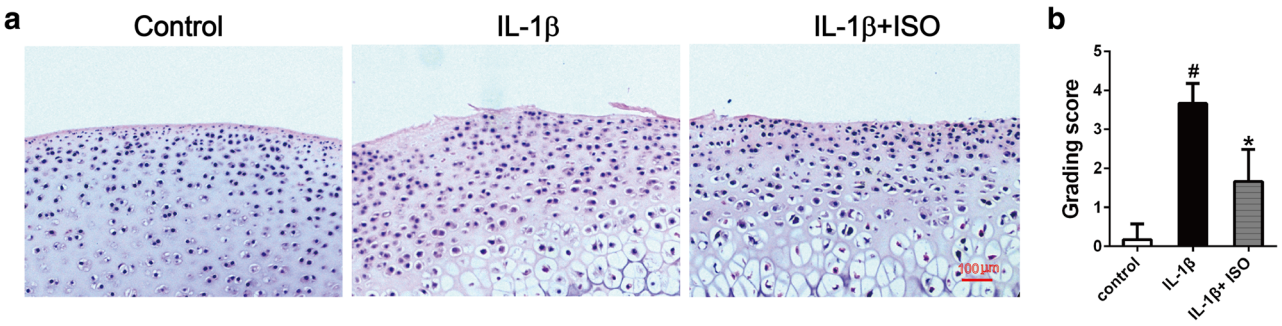


**Fig. 6.** ISO alleviated IL-1β-induced rat cartilage explants damage. (**a**) Rat cartilage explants were exposed to 20 μM ISO with or without IL-1β (10 ng/ml) for 3 days. Samples of each group were fixed, embedded, sliced coronally, and stained with HE. (**b**) Quantification analysis of the samples in each group ($n$ = 6). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* IL-1β group.

anti-inflammatory potential and low side reaction, natural plant extracts have been widely explored in OA therapy [18]. Isorhapontigenin (ISO), a natural bioavailable plant extract, had been proven to share promising anti-inflammatory potential in various diseases including chronic obstructive pulmonary disease [11] and myocardial infarction [19]. Moreover, ISO has been reported to possess favorable pharmacokinetic profiles, which indicates its potential therapy prospect [20]. Therefore, it will be interesting to investigate the exact role of ISO in OA. For the first time, we report the protective effects of ISO on IL-1β-treated rat chondrocytes *via* blocking MAPK-associated ERK and p38 pathways activation in this study (Fig. 7).

During the progression of OA, inflammatory response and cartilage degeneration is ubiquitous [21]. Accumulating studies indicated that IL-1β may serve as an important trigger in OA [6, 22]. Besides, IL-1β exhibited the most significant effects in rat chondrocytes with a concentration of 10 ng/ml [23]. Accordingly, IL-1β at this dose was employed as the stimulus in our study. A previous study confirmed that pro-inflammatory factors such as iNOS and COX2 could promote cartilage matrix damage and induce chondrocyte apoptosis [24, 25]. Moreover, increased iNOS and COX2 will further contribute to the elevated inflammatory mediators including NO and PGE2 in experimental OA [26]. Our data revealed that ISO could significantly attenuate the upregulation of iNOS, COX2, NO, and PGE2 in IL-1β-treated rat chondrocytes. Meanwhile, MMPs and ADAMTS5 are the two main catabolic enzymes which were responsible for the cartilage matrix components collagen II and aggrecan degradation, respectively [27]. In our study, ISO was also proved to inhibit the increased MMPs and ADAMTS5 in IL-1β-treated cells. Furthermore, ISO could alleviate the cartilage matrix damage both in cell and tissue experiments.

The underlying mechanism behind OA is complicated. Numerous signal pathways were involved in the OA progression. In our study, MAPK pathway was focused. MAPK pathway plays an important role in the cartilage degradation and chondrocyte inflammation [28]. Elevated levels of MMPs and ADAMTS5 are often accompanied by MAPK activation in articular cartilage [29]. Conventional MAPK signal pathway is mainly composed of ERK, p38, and JNK, which can be activated *via* phosphorylation [30]. In our study, IL-1β treatment markedly induced the phosphorylation of ERK, p38, and JNK. However, ISO

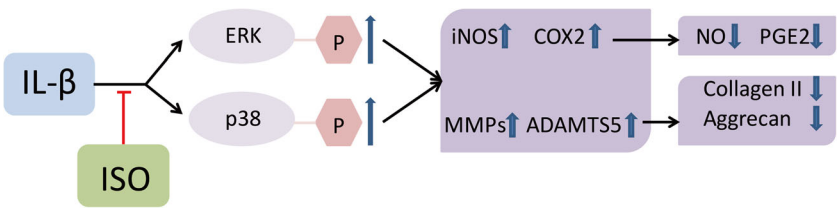


**Fig. 7.** Schematic diagram of the protective effects of ISO on IL-1β-treated rat chondrocytes. IL-1β could dramatically induce the pro-inflammatory factors including iNOS and COX2 which further trigger the upregulation of NO and PGE2 in rat chondrocytes. Besides, IL-1β promoted the synthesis of cartilage matrix catabolic enzymes such as MMPs and ADAMTS5 which is tightly related to the degradation of cartilage matrix degradation. However, ISO could reverse this change partly by blocking IL-1β-induced MAPK-associated ERK and P38 pathway activation.

administration could significantly suppress the activation of ERK and p38. Collectively, the mechanism of ISO in suppressing IL-1β-induced inflammation and cartilage damage is partly associated with blocking ERK and p38 pathways.

In view of the current study, some limitations should be noted. We fail to detect the therapeutic effects of ISO in an animal OA model. Moreover, the key target of ISO in OA progression remains elusive. Further experiments regarding these two points are needed.

In conclusion, our work firstly demonstrated that ISO suppressed inflammation and cartilage matrix damage in IL-1β-treated rat chondrocytes *via* inhibition of MAPK-associated ERK and p38 pathways. These findings may provide a potential new strategy for OA therapy.

## ACKNOWLEDGMENTS

This study was supported by the grant from the National Natural Science Foundation of China (no. 51537004) to H.W.

## COMPLIANCE WITH ETHICAL STANDARDS

This study was in strict accordance with the Guidelines of Animal Care and Use Committee for Teaching and Research of Huazhong University of Science and Technology, Wuhan, China.

**Conflict of Interest.** The authors have no conflict of interest or financial disclosures with this research.

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