Catalpol Attenuates IL-1β Induced Matrix Catabolism, Apoptosis and Inflammation in Rat Chondrocytes and Inhibits Cartilage Degeneration

Authors' Contribution:
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Data Collection B
Statistical Analysis C
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Background: Chondrocyte dysfunction and apoptosis are 2 major features during the progression of osteoarthritis. Catalpol, an iridoid glycoside isolated from the root of *Rehmannia*, is a valuable medication with anti-inflammatory, anti-oxidative, and anti-apoptotic effects in various diseases. However, whether catalpol protects against osteoarthritis has not been investigated.

Material/Methods: To assess the role of catalpol in osteoarthritis and the potential mechanism of action, chondrocytes were treated with interleukin (IL)-1β and various concentrations of catalpol. Catabolic metabolism, apoptotic level and relative signaling pathway were measured by western blot, real-time polymerase chain reaction and immunofluorescence staining. Meanwhile, we assess the cartilage degeneration in an experimental rat model using Safranin O fast green staining and cartilage was graded according to the Osteoarthritis Research Society International (OARSI) system.

Results: The results showed that catalpol prevented chondrocyte apoptotic level triggered by IL-1β, suppressed the release of catabolic enzymes, and inhibited the degradation of extracellular matrix induced by IL-1β. Catalpol also inhibited the nuclear factor kappa B (NF-κB) pathway, reduced the production of inflammatory cytokines (IL-6, tumor necrosis factor-α) in IL-1β-treated chondrocytes, and partially reversed cartilage degeneration in the knee joint in animal model of osteoarthritis.

Conclusions: Our work suggested that catalpol treatment attenuates IL-1β-induced inflammatory response and catabolism in rat chondrocytes by inhibiting the NF-κB pathway, suggesting the therapeutic potential of catalpol for the treatment of osteoarthritis.

MeSH Keywords: **Anti-Inflammatory Agents • Apoptosis • Osteoarthritis**

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Background

Osteoarthritis (OA) is one of major degenerative diseases that cause chronic pain in middle-aged to older adults around the world [1]. It is characterized by progressive degeneration of the cartilage and extracellular matrix (ECM) [2,3]. OA progression has been linked to obesity, inflammation, and mechanical stress [4,5]. The specific molecular mechanism of OA remains unclear, but it is clearly complex. Therefore, fully elucidating the pathogenesis of OA will promote the development of novel treatment strategies for this common disease.

In healthy cartilage, the chondrocyte has a very low turnover, which ensures the metabolic balance of ECM, such as aggrecan with a half-life of 3–24 years [6] and collagen with a half-life of 100 years or more [7]. By maintaining the internal pressure of the cartilage, the ECM constitutes a stable hydrodynamic system which maintains cartilage function [8]. Accordingly, chondrocytes are crucial in cartilage matrix repair. The metabolic balance of ECM is mainly controlled by catabolic enzyme produced and released by cartilage chondrocytes, including matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). Evidence of the importance of the catabolic activities in the pathogenesis of OA [9] comes from studies showing that several collagenolytic MMPs are upregulated in the chondrocytes of OA patients [10], and that there is high-level expression of aggrecanases ADAMTS-1, -4, -5, -9, and -15 in OA cartilage [11]. The excessive apoptosis of chondrocytes is another primary feature of the cartilage degeneration seen in OA [12–14] whereas the inhibition of pro-apoptotic caspase signaling attenuates OA progression [15].

Several research studies have suggested that the inflammatory response plays an essential role during the development of OA. Levels of the pro-inflammatory factors tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β are significantly upregulated in OA cartilage and mediate cartilage degeneration [16]. Macrophages and other innate immune cells are key elements in the inflammation associated with OA progression [17,18]. Macrophages sense harmful environmental conditions by targeting pathogen-associated molecular patterns or endogenous danger-associated molecular patterns. Recognition of these chemical disturbances contributes to the accumulation of inflammasomes and the subsequent release of IL-1β [19], a vital part of the cytokine network related to OA [5]. IL-1β is widely applied in studies of the pathophysiology of OA, to trigger the release of inflammatory cytokines as well as chondrocyte apoptosis [20]. Regulation of the levels of several catabolic enzymes (MMPs or ADAMTS) is achieved by transcription factors, especially nuclear factor-kappa B (NF-κB) [21,22].

Catalpol (CAT), an iridoid glycoside isolated from the root of *Rehmannia*, exerts protective effects against various diseases [23]. In a transient global ischemia model, CAT was shown to participate in the regulation of apoptosis in hippocampal CA1 neurons, by regulating the levels of Bcl-2 and Bax [24]. CAT also protects mice against renal ischemia/reperfusion injury, by regulating the PI3K/Akt-eNOS signaling and the inflammatory response [25]. Given the established anti-apoptotic and anti-inflammatory effects of CAT, the present study was designed to explore its potential protective role in a rat model of OA involving the knee joint.

The potential effects of CAT in rats were explored by assessing the apoptotic and metabolic levels of the ECM in CAT-treated chondrocytes. We also examined whether the observed attenuation of cartilage degeneration by CAT was mediated by the NF-κB pathway. Our results demonstrate the potential value of CAT as the basis of potential strategies for the prevention and treatment of OA.

Material and Methods

Reagents and antibodies

The CAT (purity >98%) was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). IL-1β was obtained from PeproTech. Primary antibodies (anti-cleaved caspase-3, anti-p65, anti-IκBα and anti-β-actin) were acquired from Cell Signaling Technology (Danvers, MA, USA), and those (anti-TNF-α and anti-IL-6) were supplied by Sigma-Aldrich (St Louis, MO, USA).

Chondrocyte culture and treatment

The knee cartilage of Sprague-Dawley (SD) rats (body weight 180–220 g) was collected for chondrocyte culture. All animal experiments were operated in compliance with the standards of the Animal Care and Use Committee of Hainan Medical University (Haikou, China). Chondrocytes, isolated and cultured as previously described [26], were used within the first 3 passages. The tissue was collected in 0.25% trypsin-EDTA for 30 minutes and then incubated with 0.2% collagenase-II for 4 hours. The isolated chondrocytes were suspended in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotic, plated in 6-well plates (1×10^5 cells) per well. After 2 days, the chondrocytes were treated for 1 day with IL-1β (10 ng/mL) with or without a combination of various concentrations of CAT (as shown in the Figures). The chondrocytes were then collected for real-time polymerase chain reaction (RT-PCR) and western blot.

Cell viability

Cell viability was monitored by Cell Counting Kit-8 (CCK-8) reagent with the manufacturer's instructions. Chondrocytes were seeded into a 96-well plate (5×10^3 cells) per well and cultured for 1 day after being treated with CAT with or without a combination of IL-1β. Then the wells were washed, and 100 µL of DMEM containing CCK-8 solution (10 µL) was added to each well and incubated for another 2 hours. The values of absorbance in each well were recorded using a microplate spectrophotometer at 450 nm.

Quantitative RT-PCR

The mRNA (2 µg) was isolated from chondrocytes using the TRIzol method using the manufacturer's protocol. Synthesis and amplification of cDNA were operated by usage of the PrimeScript RT-PCR kit and SYBR Premix Ex Taq (Roche Diagnostics GmbH, Penzberg, Germany). The gene levels were detected using the DDCt method and the value was normalized to the level of β-actin mRNA.

Western blot assay

Protein extractions from chondrocytes were performed using RIPA buffer and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on an 8–12% polyacrylamide gel. The protein (60 µg) was then transferred to previously blocked polyvinylidene difluoride membrane and probed overnight with primary antibodies (anti-TNF-α, anti-IL-6, anti-p65, anti-IκBα, anti-cleaved caspase-3, and anti-β-actin). The following day, the membrane was incubated for 1 hour with the secondary antibodies. The resulting signal was revealed and quantified by the ChemiDicTM XRS+Imaging System (Bio-Rad, Hercules, CA, USA).

Immunofluorescence staining

For collagen-II and p65 staining, the chondrocytes were plated in the glass coverslips into 6-well plates. After treatment, the slides were fixed using 4% paraformaldehyde (PFA) for 15 minutes, permeabilized in 0.1% Triton X-100 for 15 minutes, and incubated in 5% BSA for 1 hour before being treated with primary antibodies (anti-p65 and anti-collagen II). After an overnight incubation at 4°C, the specimens were treated with the secondary antibodies for 1 hour. Then slides were stained with DAPI (4',6-diamidino-2-phenylindole) for another 10 minutes. The slides were viewed and imaged by a Nikon ECLIPSE Ti microscope (Nikon, Tokyo, Japan).

Immunohistochemistry analysis

For cleaved caspase-3 staining, the specimens were fixed using 4% PFA and embedded in wax. After dewaxing and hydration, the samples were blocked by 3% H_2O_2 for 15 minutes and 5% bovine serum albumin (BSA) for 45 minutes before being treated with primary antibodies (anti-cleaved caspase-3). After an overnight incubation at 4°C, the specimens were treated with horseradish peroxidase (HRP) conjugated secondary antibody for 1 hour. Then slides were stained with hematoxylin for another 10 minutes. The slides were viewed and imaged by a light microscopy.

Model and treatment of OA

The protocol used in the animal experiments was in compliance with the standards of the Animal Care and Use Committee at Hainan Medical University. Fifteen 3-month-old male SD rats were supplied from the Animal Center of the Chinese Academy of Sciences (Shanghai, China). All rats were anesthetized via intraperitoneal injection of 2% pentobarbital (40 mg/kg). The right anterior cruciate ligament (ACL) was transected, and the right medial meniscus was resected aseptically [27]. The sham group underwent the same surgical preparation but without ACL transection or meniscus resection. After operation, animals in the OA+CAT group received CAT (5 mg/kg/day; intraperitoneal injection) [28].

Histological assay

Isolated right knee joints were fixed in 4% PFA for a day, underwent decalcification and dehydration, and then were embedded in paraffin. The specimens were cut into 4-µm thick sections for Safranin O-fast green staining to evaluate cartilage degeneration by light microscopy. The condition of the cartilage was also graded according to the Osteoarthritis Research Society International (OARSI) system [29].

Statistical analysis

Data were expressed as means ± standard error of the mean (SEM) of 5 independent experiments. A one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to for comparison between different group. A *P* value <0.05 was considered statistically significant.

Results

CAT decreased apoptosis in chondrocytes

Possible cytotoxic effects of CAT on chondrocytes were assessed by incubating the cells with various concentrations of
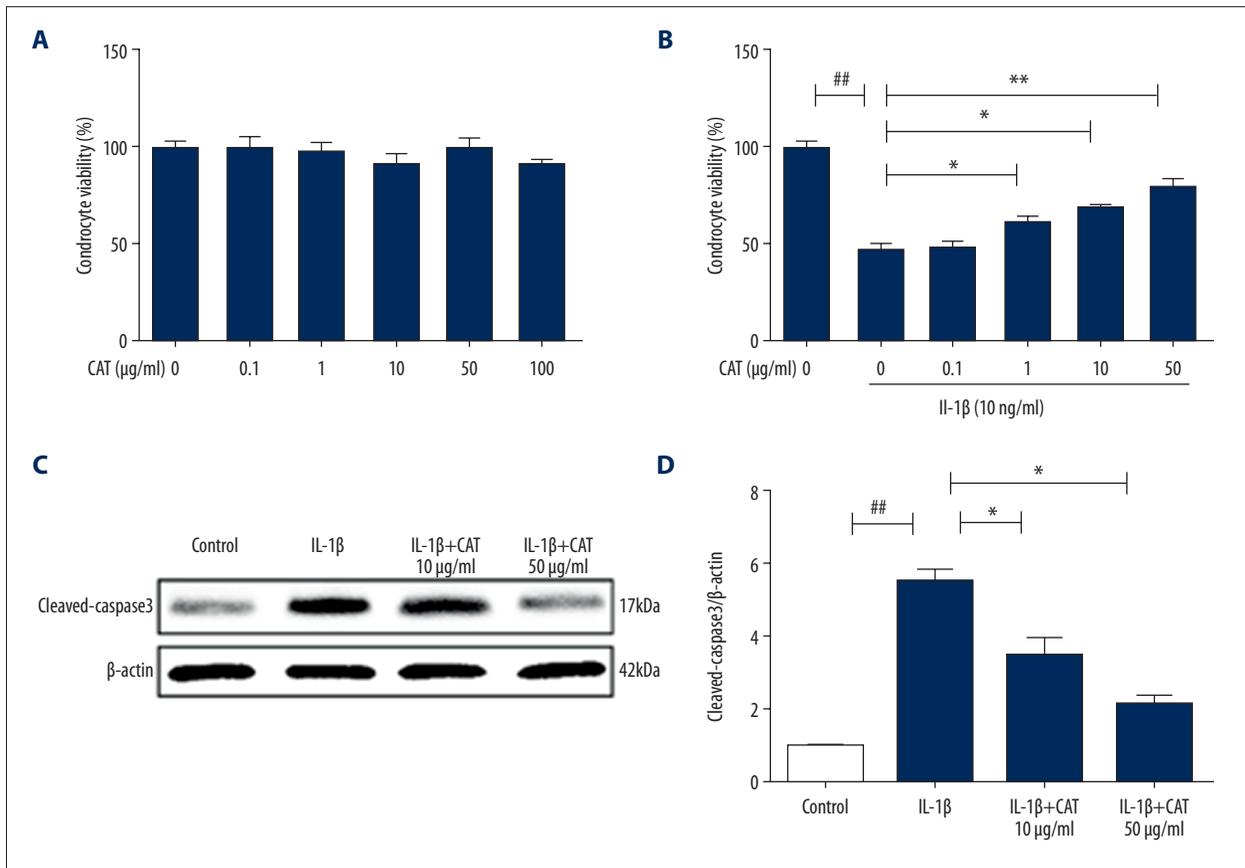
Figure 1. CAT decreases apoptotic level in rat chondrocytes. (**A**) The cytotoxic effect of CAT on chondrocytes was tested at various concentrations for 1 day using CCK-8 method. (**B**) CCK-8 assay of CAT-treated chondrocytes induced by IL-1β. (**C, D**) Western blotting and quantification analysis of cleaved caspase-3 among different groups. The values presented are the mean ± standard deviation; significant differences among control and IL-1β group are expressed as # $P<0.05$, ## $P<0.01$, ### $P<0.001$, and significant differences among CAT+IL-1β group and IL-1β groups are expressed as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n=5. CAT – catalpol; CCK-8 – Cell Counting Kit-8; IL – interleukin.

CAT for 1 day. CCK-8 assay showed that there was no chondrocyte cytotoxicity at final CAT concentrations between 0 and 100 μg/mL. Thus, within this range, CAT had no effect on chondrocyte viability (Figure 1A). Moreover, at concentrations <100 μg/mL, CAT exerted a remarkable protective effect in IL-1β-stimulated group (Figure 1B). Western blotting showed that CAT significantly reduced the expression of the pro-apoptotic protein cleaved caspase-3 in IL-1β-treated chondrocytes (Figure 1C, 1D). Together, the data revealed that CAT exerts a protective effect on chondrocytes.

CAT decreased the expressions of MMP-1, MMP-3, and MMP-13 in IL-1β-treated chondrocytes

IL-1β stimulation significantly triggered the mRNA and protein expression of MMP-1, MMP-3, and MMP-13 compared to the control group. The effect of CAT on the level of MMPs in mRNA and protein was tested by PCR and enzyme-linked immunosorbent assay (ELISA), respectively. A comparison with

chondrocytes treated with IL-1β alone showed that CAT treatment resulted in statistically significant decreases in MMPs gene and protein expression (Figure 2).

**CAT decreased the IL-1β-induced expression of ADAMTS-4 and ADAMTS-5 in chondrocytes**

IL-1β-stimulated chondrocytes treated with CAT showed dose-dependent reductions in ADAMTS-4, and ADAMTS-5 gene and protein levels compared to chondrocytes induced by IL-1β alone (Figure 3).

CAT inhibited IL-1β-mediated catabolic activity on the levels of major ECM proteins in rat chondrocytes

An immunofluorescence assay revealed a decrease in collagen II-positive staining in IL-1β-stimulated chondrocytes (Figure 4A), and that the effect was attenuated by CAT treatment. These results were confirmed by PCR, which showed that collagen II and
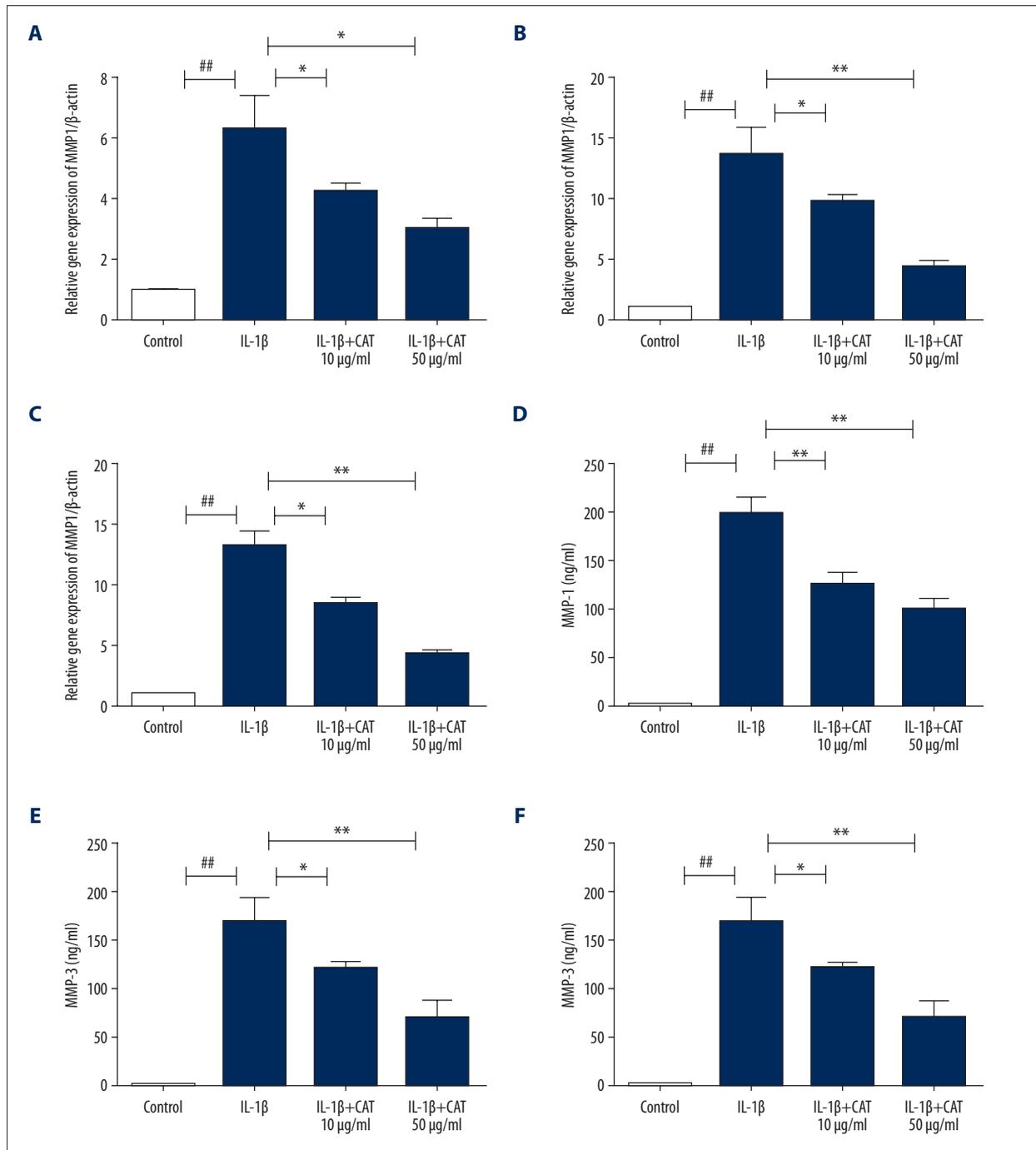
Figure 2. CAT decreases the levels of MMPs stimulated by IL-1β in chondrocytes. (**A–F**) The relative mRNA level of (**A–C**) MMP-1, MMP-3, and MMP-13 were detected by PCR. The protein level of (**D–F**) MMP-1, MMP-3, and MMP-13 were evaluated by ELISA. The values presented are the mean ± standard deviation; significant differences among control and IL-1β group are expressed as # *P*<0.05, ## *P*<0.01, ### *P*<0.001, and significant differences among CAT+IL-1β group and IL-1β groups are expressed as * *P*<0.05, ** *P*<0.01, *** *P*<0.001, n=5. CAT – catalpol; MMP – matrix metalloproteinases; IL – interleukin; PCR – polymerase chain reaction; ELISA – enzyme-linked immunosorbent assay.
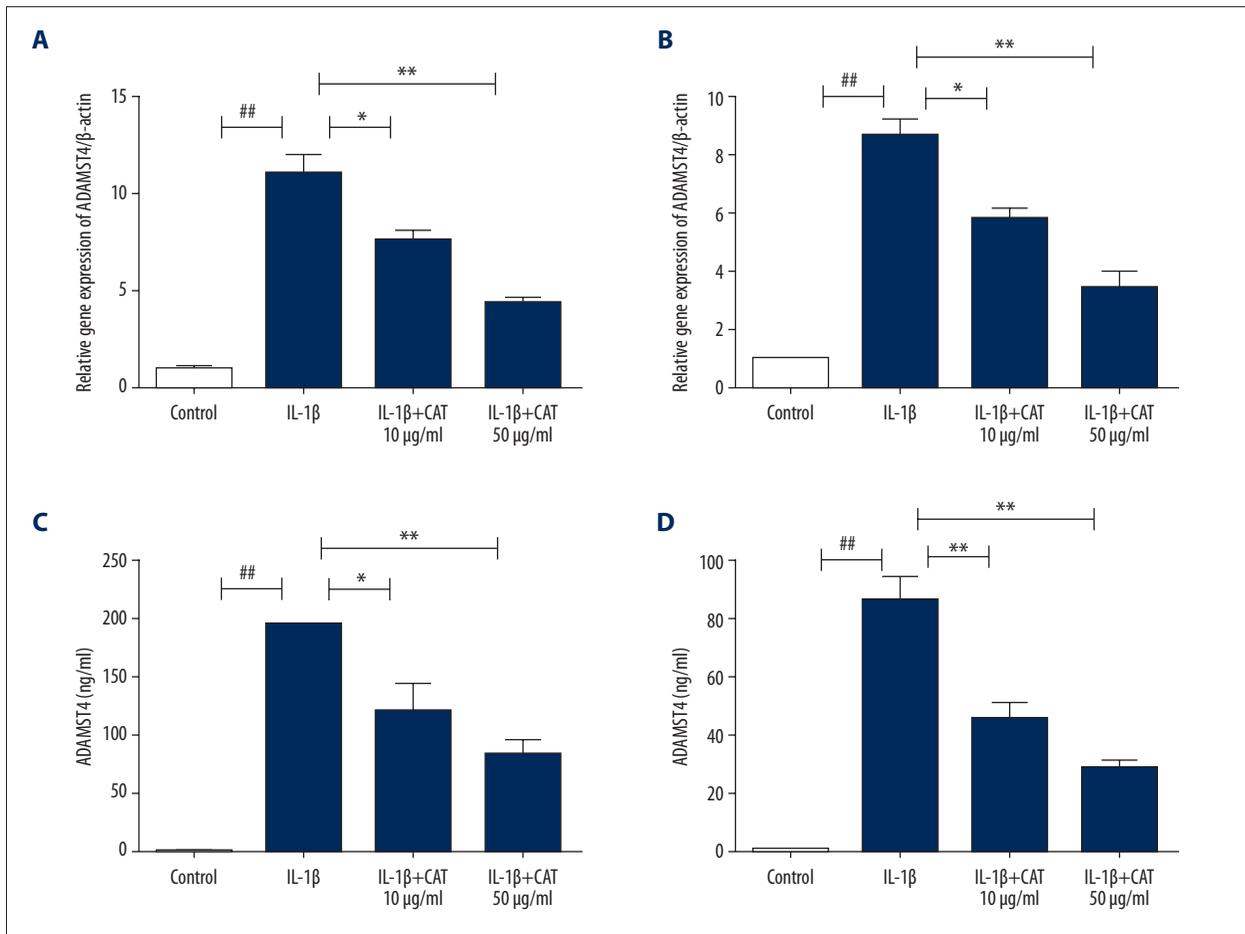
Figure 3. CAT decreases the levels of ADAMTS-4 and ADAMTS-5 stimulated by IL-1β in chondrocytes. (**A–D**) The target mRNA levels of (**A**) ADAMTS-4, (**B**) ADAMTS-5 were detected by PCR. The protein levels of (**C**) ADAMTS-4, (**D**) ADAMTS-5 were evaluated by ELISA. The values presented are the mean ± standard deviation; significant differences among control and IL-1β group are expressed as # $P<0.05$, ## $P<0.01$, ### $P<0.001$, and significant differences among CAT+IL-1β group and IL-1β groups are expressed as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n=5. CAT – catalpol; ADAMTS – a disintegrin and metalloproteinase with thrombospondin motifs; IL – interleukin; PCR – polymerase chain reaction; ELISA – enzyme-linked immunosorbent assay.

aggrecan mRNA levels were downregulated by IL-1β exposure and that CAT treatment inhibited this response (Figure 4B, 4C).

CAT decreased the IL-1β-induced release of pro-inflammatory factors in chondrocytes

Compared to control unstimulated cells, IL-1β-induced chondrocytes produced significantly larger amounts of IL-6 and TNF-α. By contrast, CAT treatment decreased the amount of IL6 and TNF-α produced by IL-1β-stimulated group (Figure 5), indicating that CAT is able to attenuate IL-1β-induced inflammation.

**CAT suppressed NF-κB pathway activation in IL-1β-stimulated chondrocytes**

To determine the molecular mechanism underlying CAT activity in IL-1β-treated chondrocytes, our work detected whether

it was related to the NF-κB pathway. The expression of p65 and IκBα protein was investigated by western blot. IκBα is the upstream signaling protein of NF-κB, and its degradation of IκBα is an indicator of the signaling pathway activation. Compared with the control group, p65 was upregulated, and IκBα downregulated, in IL-1β-treated chondrocytes, indicating enhanced activity of the NF-κB pathway (Figure 6A–6C). Immunofluorescence assays showed clear and enhanced nuclear translocation of p65 in IL-1β-induced group. Translocation was reversed by CAT (Figure 6D), indicative of its ability to suppress the NF-κB pathway in IL-1β-induced chondrocytes.

CAT attenuated apoptotic level and ECM degradation in rat cartilage of OA

The histopathological situation in both the cartilage matrix and the articular structure of the rat knee joint were evaluated
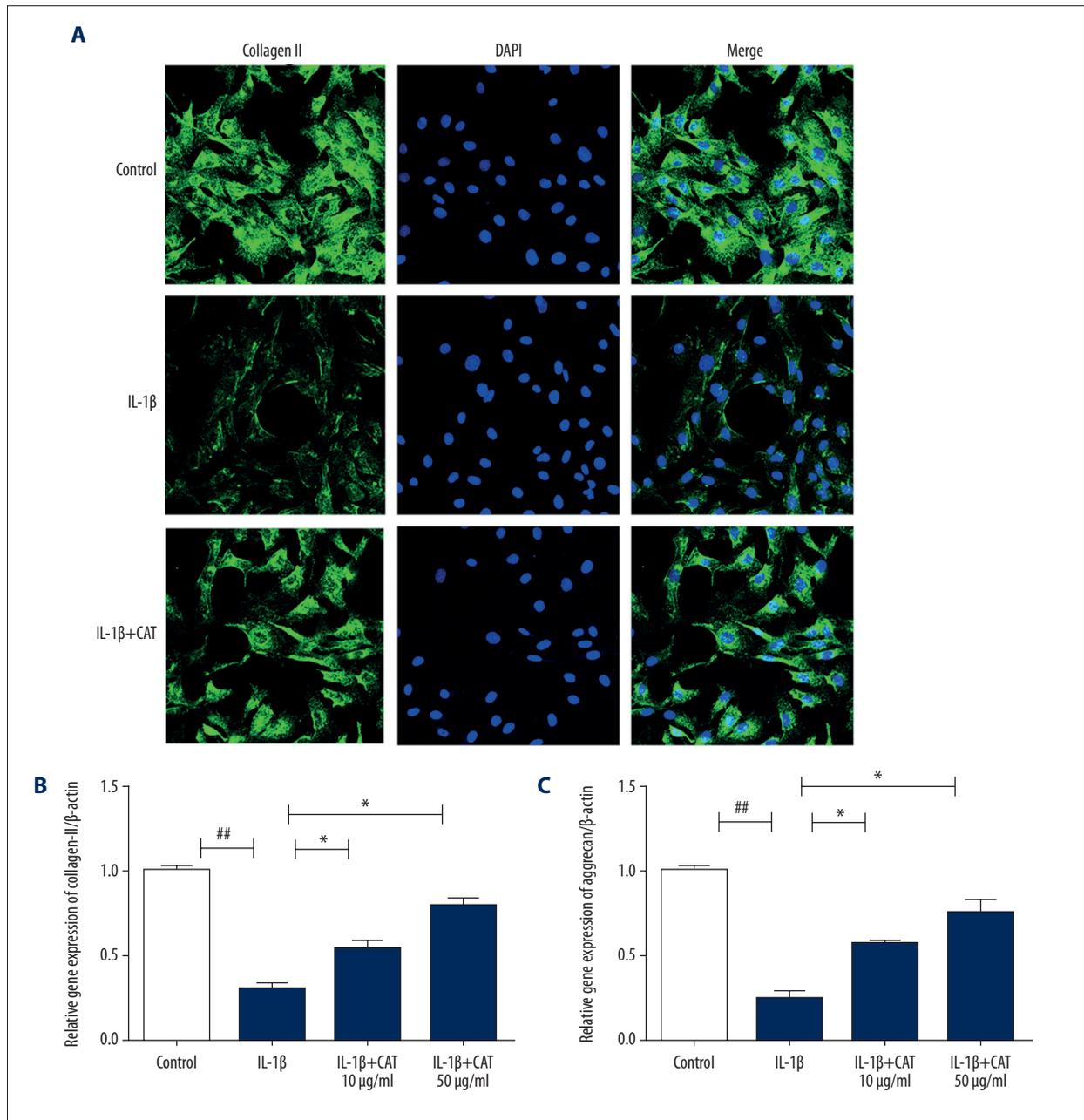
Figure 4. CAT inhibits IL-1β induced catabolic activities on the level of ECM protein in rat chondrocytes. (**A**) Immunofluorescence assay of collagen II protein (green) and nucleus (blue). (**B, C**) The relative mRNA levels of (**B**) collagen II, (**C**) aggreacan were evaluated by PCR. The values presented are the mean ± standard deviation; significant differences among control and IL-1β group are expressed as # $P<0.05$, ## $P<0.01$, ### $P<0.001$, and significant differences among CAT+IL-1β group and IL-1β groups are expressed as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n=5. CAT – catalpol; IL – interleukin; ECM – extracellular matrix; PCR – polymerase chain reaction; ELISA – enzyme-linked immunosorbent assay.

using Safranin O staining and quantified using the OARSI scoring system. The articular cartilage in the OA group exhibited remarkable degeneration, including cartilage erosion, as well as loss of ECM proteins and cells. The ability of CAT to considerably attenuate cartilage destruction in the OA group was supported by the OARSI score (Figure 7A, 7B). In the immunohistochemical assay, CAT clearly reduced the level of cleaved caspase-3 in the articular cartilage of the OA group (Figure 7C), consistent with the results of the *in vitro* experiments. These results demonstrated the significant protective effects of CAT *in vivo*.
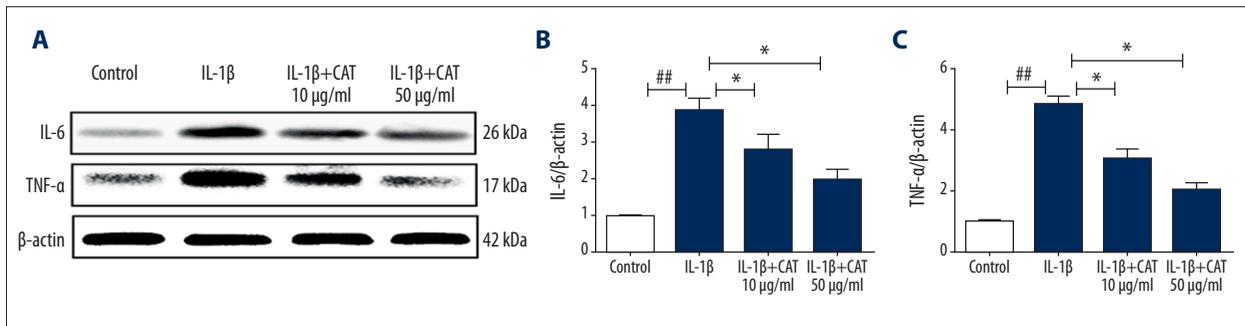
Figure 5. CAT attenuates the release of pro-inflammatory factors induced by IL-1β in rat chondrocytes. (**A–C**) Western blotting and quantification analysis of IL-6 and TNF-α among different groups. The values presented are the mean ± standard deviation; significant differences among control and IL-1β group are expressed as # $P<0.05$, ## $P<0.01$, ### $P<0.001$, and significant differences among CAT+IL-1β group and IL-1β groups are expressed as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n=5. CAT – catalpol; IL – interleukin; TNF – tissue necrosis factor.
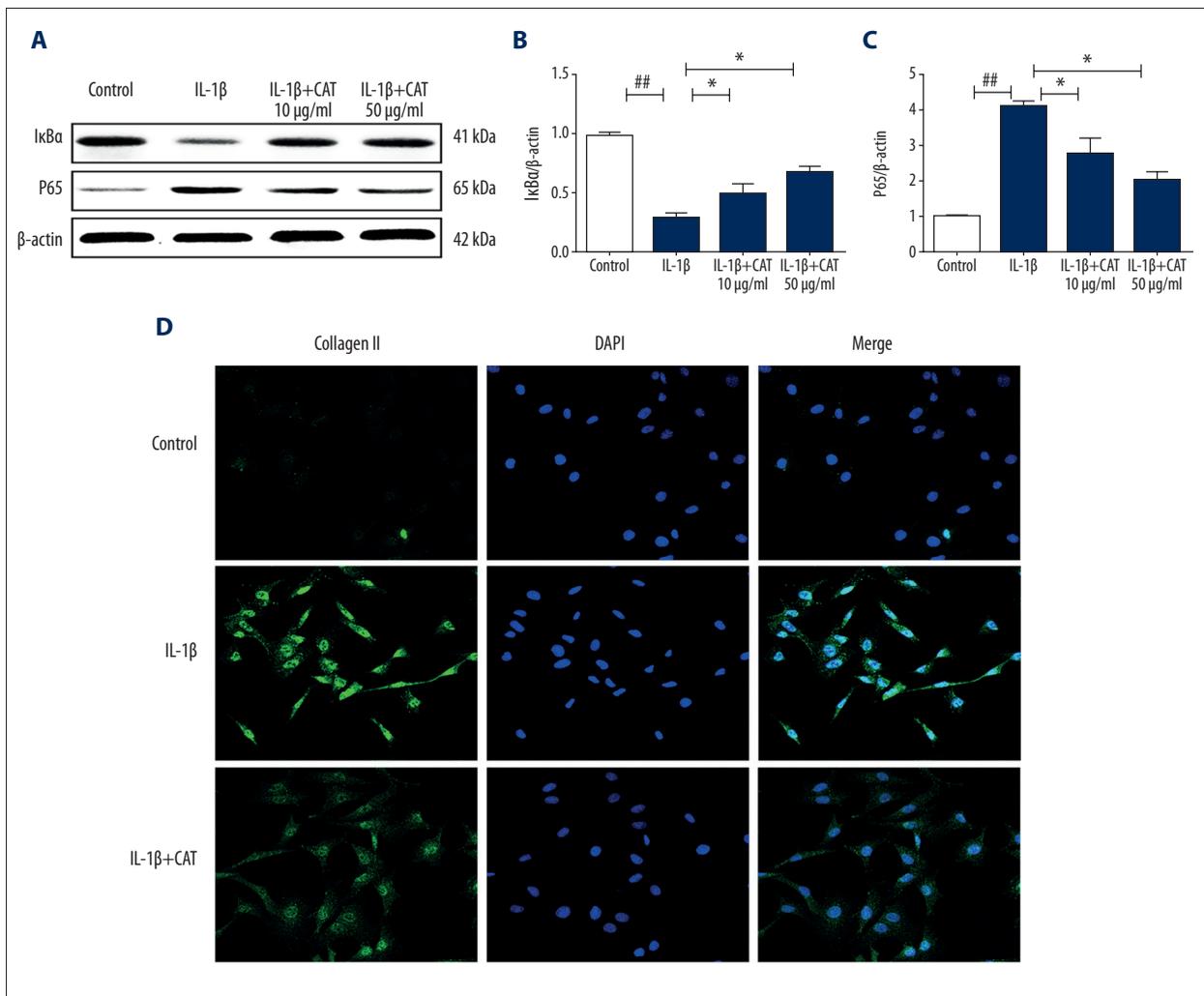
Figure 6. CAT suppressed NF-κB pathway in chondrocytes treated with IL-1β. (**A–C**) Western blotting and quantification analysis of p65 and IκBα among different groups. (**D**) Immunofluorescent assay of p65 (green) and nucleus (blue). The values presented are the mean ± standard deviation; significant differences among control and IL-1β group are expressed as # $P<0.05$, ## $P<0.01$, ### $P<0.001$, and significant differences among CAT+IL-1β group and IL-1β groups are expressed as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n=5. CAT – catalpol; NF-κB – nuclear factor kappa B; IL – interleukin.
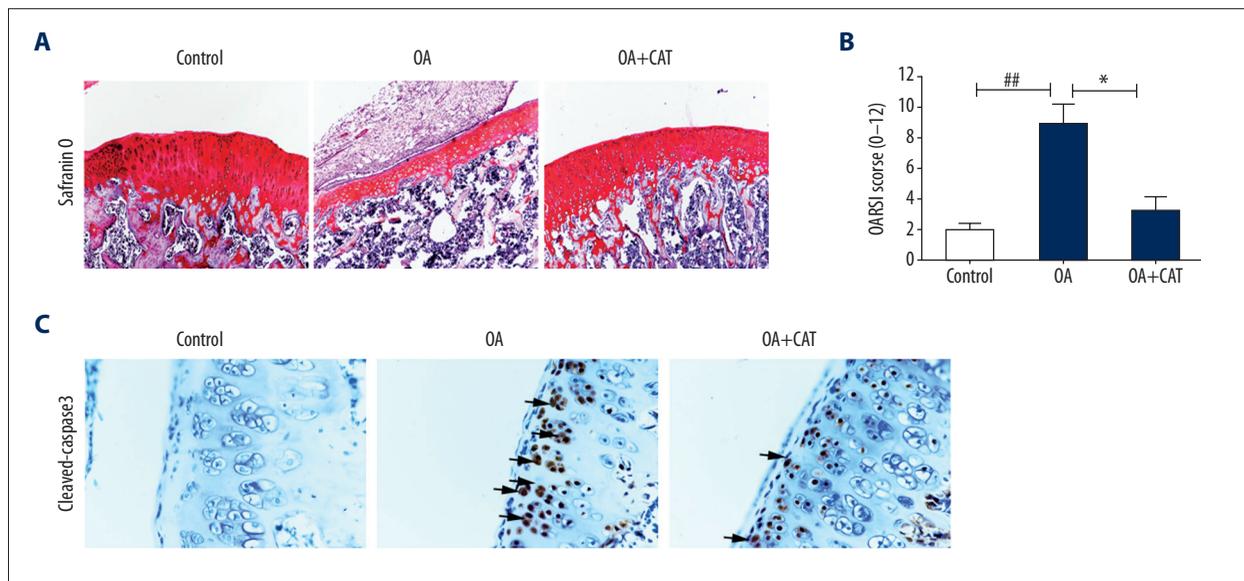
Figure 7. (A–C) CAT attenuates apoptotic level and ECM degradation in rat cartilage of OA. (**A**) Representative Safranin O staining of the cartilage from each group at 8 weeks post-operation. (**B**) OARSI scores among different groups at 8 weeks. The values presented are the mean ± standard deviation; significant differences among sham and OA group are expressed as # *P*<0.05, ## *P*<0.01, ### *P*<0.001, and significant differences among OA group and CAT+OA group are expressed as * *P*<0.05, ** *P*<0.01, *** *P*<0.001, n=5. CAT – catalpol; ECM – extracellular matrix; OA – osteoarthritis; OARSI – Osteoarthritis Research Society International.

Discussion

OA is one of major degenerative joint diseases characterized by the dysfunction and excess apoptosis of chondrocytes. Genetic and environmental factors, as well as aging, play a role in its pathogenesis [30,31]. The reduction of chondrocytes results in the absence of several ECM proteins, and therefore cartilage matrix degradation [32,33]. An increase in apoptosis in human chondrocytes from normal and OA cartilage was shown to be related to the inflammatory response of the cells [34,35]. Several intracellular signaling pathways may exacerbate cartilage destruction by stimulating the secretions of catabolic enzymes, including MMP-1, MMP-2, and MMP-3, and other catabolic factors, including COX-2, nitric oxide and nitric oxide synthase, which enhance apoptotic level in OA chondrocytes and thereby the degradation of cartilage ECM [14,36]. New treatment strategies that reduce chondrocyte apoptosis and promote chondrocyte function are therefore vital.

The catabolic enzymes that are upregulated in IL-1β-induced chondrocytes include MMPs (MMP-1, MMP-3, and MMP-13), ADAMTS-4, and ADAMTS-5 [37]. Increases in MMP-3 and MMP-13 in the cartilage are a vital feature of the pathologic progression of OA. These 2 enzymes degrade a wide array of ECM proteins, including collagen II and other proteoglycans, and promote the release of other MMPs [38,39]. A lentivirus-mediated siRNA targeting ADAMTS-5 inhibited cartilage degeneration *in vivo* experiments [27]. CAT is an iridoid

glycoside isolated from the root of *Rehmannia*, exerting protective effects against various diseases [23]. Recent studies have shown the value of CAT based on its anti-inflammatory, anti-oxidative, as well as anti-apoptotic activities, both *in vitro* and *in vivo* [40–42]. CAT protects rat embryonic ventricular myocardial cells against hydrogen-peroxide-induced apoptosis, via regulating the mitochondrial-dependent caspase pathway [43]. In this study, IL-1β increased chondrocyte apoptosis by enhancing cellular cleaved caspase-3 levels, whereas CAT had a concentration-dependent anti-apoptotic effect. Similarly, *in vivo* experiments, the expression of cleaved caspase-3 was increased in cells, whereas CAT partially reversed this effect. In addition, our work demonstrated that CAT significantly inhibited MMP-1, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5 mRNA and protein expression, in addition to reducing the degradation of collagen II and aggrecan from the ECM in IL-1β-treated group. In the CAT group, the deposition of proteoglycans in cartilage was markedly higher, and the OARSI score was lower. Together, these results provide strong evidence that the protective effects exerted by CAT treatment include a reduction in chondrocyte apoptosis and restoration of the function of these cells.

The pro-inflammatory cytokines IL-6, 8, IL-1β, and TNF-α play critical roles in the development of OA, and their suppression is a primary treatment strategy in OA [44]. Two major pro-inflammatory factors IL-1β and TNF-α have been implicated in synovitis and were shown to activate chondrocytes, resulting in apoptosis and increased expression of genes encoding

inflammatory factors, such as COX-2 and MMPs, both of which are essential to the initiation and progression of OA [45,46]. IL-1β and TNF-α recruit death-related compounds through combination with specific ligands. The result is activation of the apoptotic pathway and thus internucleosomal DNA fragmentation [20,47]. The inflammatory response exerts critical effects on metabolic processes in the ECM. For example, the inflammation-related upregulation of MMPs leads to progressive matrix catabolism [48]. CAT treatment effectively inhibited the IL-1β-induced expression of pro-inflammatory factors.

The involvement of several molecular signaling pathways during the pathophysiology of OA has been reported, and investigations into the mechanisms of OA affecting cartilage chondrocytes are a research hotspot. Members of the NF-κB family are widely expressed transcription factors regulating immunity, cell proliferation, inflammation, apoptosis, and other cellular processes [49]. Disturbances caused by harmful factors induce nuclear translocation of the phosphorylated p65 subunit of NF-κB, followed by binding to specific genes and the triggering of their transcription [50]. In chondrocytes during the progression of OA, these genes include those encoding several major catabolic enzymes (MMP-1, MMP-2, and MMP-3) and catabolic factors (COX-2 and NO) [36]. Thus, NF-κB signaling pathway is required for OA progression. Consequently, therapeutic agents targeting the NF-κB pathway will attenuate the inflammatory response of chondrocytes in OA [51]. For example, IκB inducible kinase and IκBα are key members of the NF-κB family, and their repression was shown to attenuate cartilage degeneration [52]. In our study, CAT treatment dose-dependently attenuated the level of p65 and the degradation of IκBα, indicating that CAT inhibited the NF-κB pathway. Furthermore, in the immunofluorescence assay, CAT prevented the nuclear translocation of p65, consistent with the results of the western blot.

Conclusions

Our work suggested that CAT treatment attenuates IL-1β-induced inflammatory response and apoptotic level in rat chondrocytes by inhibiting the NF-κB pathway. The result was CAT-mediated suppression of ECM catabolism in OA chondrocytes. *In vivo* experiments also suggested that CAT prevents cartilage degeneration. Our study thus provides important new insights into the mechanisms underlying the protective effect of CAT, as well as support for further studies of CAT in the treatment of OA.

Conflict of interest

None.

References:

1. Wilcox S, Brenes GA, Levine D et al: Factors related to sleep disturbance in older adults experiencing knee pain or knee pain with radiographic evidence of knee osteoarthritis. J Am Geriatr Soc, 2000; 48: 1241–51

2. Aigner T, Kim HA: Apoptosis and cellular vitality: Issues in osteoarthritic cartilage degeneration. Arthritis Rheum, 2002; 46: 1986–96

3. Zhang HB, Zhang Y, Chen C et al: Pioglitazone inhibits advanced glycation end product-induced matrix metalloproteinases and apoptosis by suppressing the activation of MAPK and NF-kappaB. Apoptosis, 2016; 21: 1082–93

4. Yang Y, Wang Y, Kong Y et al: Mechanical stress protects against osteoarthritis via regulation of the AMPK/NF-kappaB signaling pathway. J Cell Physiol, 2019; 234(6): 9156–67

5. Yazigi F, Espanha M, Marques A et al: Predictors of walking capacity in obese adults with knee osteoarthritis. Acta Reumatol Port, 2018; 43: 256–63

6. Maroudas A, Bayliss MT, Uchitel-Kaushansky N et al: Aggrecan turnover in human articular cartilage: Use of aspartic acid racemization as a marker of molecular age. Arch Biochem Biophys, 1998; 350: 61–71

7. Verzijl N, DeGroot J, Oldehinkel E et al: Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochem J, 2000; 350(Pt 2): 381–87

8. Matyas JR, Adams ME, Huang D et al: Discoordinate gene expression of aggrecan and type II collagen in experimental osteoarthritis. Arthritis Rheum, 1995; 38: 420–25

9. Huang X, Pan Q, Mao Z et al: Kaempferol inhibits interleukin-1beta stimulated matrix metalloproteinases by suppressing the MAPK associated ERK and P38 signaling pathways. Mol Med Rep, 2018; 18: 2697–704

10. Smith GN Jr.: The role of collagenolytic matrix metalloproteinases in the loss of articular cartilage in osteoarthritis. Front Biosci, 2006; 11: 3081–95

11. Nagase H, Kashiwagi M: Aggrecanases and cartilage matrix degradation. Arthritis Res Ther, 2003; 5: 94–103

12. Rowan AD: Cartilage catabolism in arthritis: Factors that influence homeostasis. Expert Rev Mol Med, 2001; 2001: 1–20

13. Goggs R, Carter SD, Schulze-Tanzil G et al: Apoptosis and the loss of chondrocyte survival signals contribute to articular cartilage degradation in osteoarthritis. Vet J, 2003; 166: 140–58

14. Ou Y, Tan C, An H et al: Selective COX-2 inhibitor ameliorates osteoarthritis by repressing apoptosis of chondrocyte. Med Sci Monit, 2012; 18: BR247–52

15. D'Lima D, Hermida J, Hashimoto S et al: Caspase inhibitors reduce severity of cartilage lesions in experimental osteoarthritis. Arthritis Rheum, 2006; 54: 1814–21

16. Kapoor M, Martel-Pelletier J, Lajeunesse D et al: Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol, 2011; 7: 33–42

17. Orlowsky EW, Kraus VB: The role of innate immunity in osteoarthritis: When our first line of defense goes on the offensive. J Rheumatol, 2015; 42: 363–71

18. Daghestani HN, Pieper CF, Kraus VB: Soluble macrophage biomarkers indicate inflammatory phenotypes in patients with knee osteoarthritis. Arthritis Rheumatol, 2015; 67: 956–65

19. Latz E, Xiao TS, Stutz A: Activation and regulation of the inflammasomes. Nat Rev Immunol, 2013; 13: 397–411

20. Csaki C, Mobasheri A, Shakibaei M: Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: Inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. Arthritis Res Ther, 2009; 11: R165

21. Hu PF, Sun FF, Jiang LF et al: Paeoniflorin inhibits IL-1beta-induced MMP secretion via the NF-kappaB pathway in chondrocytes. Exp Ther Med, 2018; 16: 1513–19

22. Zhuang Z, Ye G, Huang B: Kaempferol Alleviates the interleukin-1beta-induced inflammation in rat osteoarthritis chondrocytes via suppression of NF-kappaB. Med Sci Monit, 2017; 23: 3925–31

23. Hu L, Sun Y, Hu J: Catalpol inhibits apoptosis in hydrogen peroxide-induced endothelium by activating the PI3K/Akt signaling pathway and modulating expression of Bcl-2 and Bax. Eur J Pharmacol, 2010; 628: 155–63

24. Li DQ, Duan YL, Bao YM et al: Neuroprotection of catalpol in transient global ischemia in gerbils. Neurosci Res, 2004; 50: 169–77

25. Zhu J, Chen X, Wang H et al: Catalpol protects mice against renal ischemia/reperfusion injury via suppressing PI3K/Akt-eNOS signaling and inflammation. Int J Clin Exp Med, 2015; 8: 2038–44

26. Jiang LB, Lee S, Wang Y et al: Adipose-derived stem cells induce autophagic activation and inhibit catabolic response to pro-inflammatory cytokines in rat chondrocytes. Osteoarthritis Cartilage, 2016; 24: 1071–81

27. Chu X, You H, Yuan X et al: Protective effect of lentivirus-mediated siRNA targeting ADAMTS-5 on cartilage degradation in a rat model of osteoarthritis. Int J Mol Med, 2013; 31: 1222–28

28. Cai QY, Chen XS, Zhan XL et al: Protective effects of catalpol on oligodendrocyte death and myelin breakdown in a rat model of chronic cerebral hypoperfusion. Neurosci Lett, 2011; 497: 22–26

29. Kraus VB, Huebner JL, DeGroot J et al: The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the guinea pig. Osteoarthritis Cartilage, 2010; 18(Suppl. 3): S35–52

30. Loeser RF: Aging and osteoarthritis: The role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthritis Cartilage, 2009; 17: 971–79

31. Brandi ML, Gennari L, Cerinic MM et al: Genetic markers of osteoarticular disorders: Facts and hopes. Arthritis Res, 2001; 3: 270–80

32. Song B, Song H, Wang W et al: Beclin 1 overexpression inhibits chondrocyte apoptosis and downregulates extracellular matrix metabolism in osteoarthritis. Mol Med Rep, 2017; 16: 3958–64

33. Gao G, Ding H, Zhuang C et al: Effects of hesperidin on H(2)O(2)-treated chondrocytes and cartilage in a rat osteoarthritis model. Med Sci Monit, 2018; 24: 9177–86

34. Heraud F, Heraud A, Harmand MF: Apoptosis in normal and osteoarthritic human articular cartilage. Ann Rheum Dis, 2000; 59: 959–65

35. Zhu X, Wang L, Teng X et al: N-Methyl pyrrolidone (NMP) alleviates lipopolysaccharide (LPS)-induced inflammatory injury in articular chondrocytes. Med Sci Monit, 2018; 24 6480–88

36. Rigoglou S, Papavassiliou AG: The NF-kappaB signalling pathway in osteoarthritis. Int J Biochem Cell Biol, 2013; 45: 2580–84

37. Zheng W, Tao Z, Cai L et al: Chrysin attenuates IL-1beta-induced expression of inflammatory mediators by suppressing NF-kappaB in human osteoarthritis chondrocytes. Inflammation, 2017; 40: 1143–54

38. Eguchi T, Kubota S, Kawata K et al: Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene. Mol Cell Biol, 2008; 28: 2391–413

39. Wang J, Ma J, Gu JH et al: Regulation of type II collagen, matrix metalloproteinase-13 and cell proliferation by interleukin-1beta is mediated by curcumin via inhibition of NF-kappaB signaling in rat chondrocytes. Mol Med Rep, 2017; 16: 1837–45

40. Hu LA, Sun YK, Zhang HS et al: Catalpol inhibits apoptosis in hydrogen peroxide-induced cardiac myocytes through a mitochondrial-dependent caspase pathway. Biosci Rep, 2016; 36(3): pii: e00348

41. Zhou J, Xu G, Ma S et al: Catalpol ameliorates high-fat diet-induced insulin resistance and adipose tissue inflammation by suppressing the JNK and NF-kappaB pathways. Biochem Biophys Res Commun, 2015; 467: 853–58

42. Zhang XL, Zhang AH, Jiang B et al: Further pharmacological evidence of the neuroprotective effect of catalpol from *Rehmannia glutinosa*. Phytomedicine, 2008; 15: 484–90

43. Hu LA, Sun YK, Zhang HS et al: Catalpol inhibits apoptosis in hydrogen peroxide-induced cardiac myocytes through a mitochondrial-dependent caspase pathway. Biosci Rep, 2016; 36: pii: e00348

44. Shen S, Guo J, Luo Y et al: Functional proteomics revealed IL-1beta amplifies TNF downstream protein signals in human synoviocytes in a TNF-independent manner. Biochem Biophys Res Commun, 2014; 450: 538–44

45. Heinegard D, Saxne T: The role of the cartilage matrix in osteoarthritis. Nat Rev Rheumatol, 2011; 7: 50–56

46. Sellam J, Berenbaum F: The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol, 2010; 6: 625–35

47. Kim HA, Blanco FJ: Cell death and apoptosis in ostearthritic cartilage. Current Drug Targets, 2007; 8: 333–45

48. Kapoor M, Martel-Pelletier J, Lajeunesse D et al: Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol, 2011; 7: 33–42

49. Oeckinghaus A, Ghosh S: The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol, 2009; 1(4): a000034

50. Perkins ND: The diverse and complex roles of NF-kappaB subunits in cancer. Nat Rev Cancer, 2012; 12: 121–32

51. Gao SG, Yu Y, Zeng C et al: Phosphorylation of osteopontin has proapoptotic and proinflammatory effects on human knee osteoarthritis chondrocytes. Exp Ther Med, 2016; 12: 3488–94

52. Boyault S, Bianchi A, Moulin D et al: 15-deoxy-delta(12,14)-prostaglandin J(2) inhibits IL-1 beta-induced IKK enzymatic activity and I kappa B alpha degradation in rat chondrocytes through a PPAR gamma-independent pathway. Febs Lett, 2004; 572: 33–40