Gigantol Alleviates IL-1β-Induced Inflammation and Catabolism in Mouse Osteoarthritis via PI3K/Akt/NF-κB Pathways In Vivo and In Vitro

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Abstract

Osteoarthritis (OA), a prevalent disabling disease, is characterized by irreversible cartilage degradation and persistent inflammation. The etiology as well as pathogenesis of OA are not completely unclear and need further investigation. Gigantol, is a bibenzyl derivative extracted from Dendrobium plants and has been found exhibit multiple effects such as anti-inflammatory effects. Nevertheless, the biological function of gigantol on osteoarthritis (OA) is still uncertain. This study aimed at examining the anti-inflammatory effects and latent mechanisms of gigantol in IL-1β-mediated OA progression. In vitro, we identified that gigantol treatment suppressed tumor necrosis factor-alpha (TNF-α), nitric oxide (NO), cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), inducible nitric oxide synthase (iNOS) and interleukin-6 (IL-6) in interleukin-1 beta (IL-1β) mediated mouse OA chondrocytes. Gigantol was also shown to dose dependently downregulate the metalloproteinase 13 (MMP13) as well as thrombospondin motifs 5 (ADAMTS5) levels. Moreover, IL-1β-mediated AKT and PI3K phosphorylation as well as NF-κB activation were inhibited by gigantol. Meanwhile, in vivo, we detected that gigantol treatment inhibited degradation of the cartilage degradation and lowered the Osteoarthritis Research Society International scores (OARSI) in OA mouse. Therefore, gigantol is a promising therapeutic option for OA.

1. Introduction

Osteoarthritis (OA), an extremely common degenerative disease of the joints, causes to joint pain and disability and has no effective therapy to date. Previous studies have demonstrated that a number of causes such as aging, trauma, inflammation, obesity, metabolic disorders, immune factors, and genetic factors are all associated with OA. However, the main molecular mechanism associated OA are currently unknown. Pathologically, the progressive destruction of articular cartilage, subchondral bone reconstruction, as well as synovial inflammation are characteristics of OA. Furthermore, increasing evidences have established that many inflammatory cytokines and mediators are deeply entailed in the development of OA. Interleukin-1β, as a vital inflammatory factor, is key in OA initiation and progression. IL-1β induces catabolic as well as pro-inflammatory factor secretion, including prostaglandin E2 (PGE2), thrombospondin motifs (ADAMTS), matrix metalloproteinases (MMPs), and nitric oxide (NO). Besides, the increase of the IL-1β would destroy the extracellular matrix (ECM) component, including collagen-II and aggrecan. The imbalance between extracellular matrix synthesis and decomposition would further accelerates OA deterioration. Consequently, the strategy targeted at IL-1β is probably feasible and effective for OA treatment.

Wide evidences have demonstrated IL-1β plays a role of catabolic effects in OA through activation of the nuclear factor kappa B (NF-κB) pathway. As a family of inducible transcription factors, NF-κB is composed by p50, p52, p65, RelB and c-Rel structurally. In the inactive state, p65 binds to a protein called IkappaB and stays in the cytoplasm. Classical NF-κB pathway activation begins with degradation of IkBα triggered by phosphorylated multi-subunit IkB kinase (IKK) complex. Without the combination of IkBα, p65 would be connected by p50 and transferred into the nucleus which could make
a difference to gene transcription. A well-recognized function of the activated NF-κB is regulation of inflammatory responses including contributing to IL-1β up-regulating MMPs and overproducing inducible nitric oxide synthase (iNOS). As researches progressed, respectable studies have discovered NF-κB could be activated directly by the phosphoinositide-3-kinase/protein kinase B (PI3K/AKT) pathway. PI3K, consisting of a catalytic (p110α) and regulatory (p85α) subunit, binds to the PH domain of AKT and induces the phosphorylation of AKT. And eventually, the phosphorylated AKT migrates to the cell membrane from the cytoplasm and ignites its downstream molecular proteins such as NF-κB. Hence, therapy effecting PI3K/Akt/NF-κB pathway suppression may stand a good chance of lessening inflammatory response caused by IL-1β in OA.

Gigantol (C_{16}H_{18}O_{4}), is a bibenzyl derivative extracted from traditional Chinese herb Dendrobium plants. Currently, a great number of studies implies that gigantol could exert multiple biological effects e.g., antioxidant, benefit of diabetic nephropathy, and restraint some kinds of cancers. Moreover, previous evidences indicated that crude extracts from Dendrobium plants motivate the production of collagen in human dermal fibroblasts and inhibited MMP. Deciga-Campos, M. et al. suggested gigantol decreased carrageenan induced inflammation in rats. In addition, previous evidence implied that gigantol can inactivate NF-κB to decrease iNOS and COX-2 levels, which could compound vital inflammatory mediators. What's more, in the human liver cancer research, gigantol has been found directly decrease PI3K/Akt/NF-κB signaling pathway activation to ameliorate human liver cancer progression. Although gigantol has been found with a wide range of pharmacological activities, the exact role of gigantol in OA is unclear yet and needs to be further studied.

Thus, we evaluated the anti-inflammatory effects and underlying mechanisms of gigantol on IL-1β-mediated mouse chondrocytes.

2. Materials And Methods

2.1. Chemicals and reagents

Gigantol (purity ≥ 98%) was bought from Sigma-Aldrich (St Louis, MO, USA). Dimethylsulfoxide (DMSO), collagenase type II, and recombinant human IL-1β were purchased from Sigma Chemical Co. (St. Louis, MO, United States). The Cell-Counting Kit-8 (CCK-8) was bought from Dojindo (Kumano, Japan). Inducible nitric oxide synthase (iNOS) antibody was purchased from Sigma-Aldrich (St Louis, MO, United States). Goat anti-mouse and anti-rabbit IgG-HRP were obtained from Bioworld (OH, United States). Primary antibodies against GAPDH and Lamin B1 were bought from Abcam (CA, United Kingdom). 4′,6-diamidino-2-phenylindole (DAPI) was bought from Beyotime (Shanghai, China). Primary antibodies for P-Akt, P-PI3K, Akt, PI3K, IkBα, COX-2, as well as p65 were purchased from CST (MA, United States). Cell culture chemicals were obtained from Gibco (Grand Island, NY, United States). Secondary antibodies (Alexa Fluor®488 and Alexa Fluor®594 labeled Goat Anti-Rabbit IgG (H + L)) were acquired from Jackson ImmunoResearch (West Grove, PA, United States)
2.2. Primary osteoarthritis chondrocyte culture

In vitro, C57BL/6 mice were anesthetized using sodium pentobarbital and sacrificed to prepare chondrocytes. We extracted knee cartilage under aseptic conditions and dissolved them at 37°C in collagenase II (0.1%) for 4 hours, followed by centrifugation at 1000rpm for 5 min. Then, extracted cells were seeded in micro-well plates containing DMEM/F12 with fetal bovine serum (FBS, 10%) and streptomycin/penicillin (1%). Incubation was done in a 5% CO2 environment at 37°C. Next, to reach 80–90% confluence, cell passaging was done using Trypsin-EDTA (0.25%). For avoiding phenotype loss, only passages 1 to 3 were adopted.

2.3. Animal models

Sixty C57BL/6 wild-type (WT) male mice (ten-week-old) were obtained from the Animal Center of the Chinese Academy of Sciences, Shanghai, China. Study approval was obtained from the Animal Care and Use Committee of Wenzhou Medical University. As previously described, mice OA surgical induction was achieved by the destabilization of medial meniscus (DMM). First, mice were randomized into 3 treatment groups, vehicle, sham, and gigantol groups. After anestheticization through peritoneal injection of pentobarbital (3%; 1 mL/kg), joint capsules of mice right knees were incised and their medial meniscotibial ligaments transected using microsurgical scissors. An arthrotomy was also conducted in the sham group, but without medial meniscus ligament transection.

2.4. Experimental design

Cells were exposed to IL-1β (10 ng/ml) either alone or when combined with gigantol pretreatment at various concentrations (10, 20 or 40 µM). Apart from medium changes, the control group was untreated. Cell harvesting was done after 24 h of incubation. Inflammatory responses in chondrocytes were sufficiently induced by 24 h of exposure to IL-1β.

In vivo study, as described above, we performed surgical DMM in mice. Gigantol was dissolved in 0.1% DMSO. Then, the gigantol treatment groups were orally treated with gigantol (25 mg/kg) every day for 8 successive weeks. The DMM alone groups were treated with an equal volume of 0.1% DMSO (physiological saline). Cartilage tissue samples were collected after eight weeks post-surgery for iconographic as well as histological evaluations.

2.5. Cell viability assay

Gigantol cytotoxicity on chondrocyts was assessed by cell counting kit-8 (CCK-8; Dojindo Co, Kumamoto, Japan). First-passage chondrocytes were cultivated in 96-well plates (50000 cells/cm2), incubated for 24 h and treated for 24 and 48 h with varying gigantol doses (0, 10, 20, 40, 60 or 100 µM). At indicated timepoints, cells were washed using phosphate buffered saline (PBS), DMEM/ F12 (100 µl) containing the CCK-8 solution (10 µl) was supplemented to every well followed by 2 h of incubation at 37°C. Absorbance was spectrophotometrically (Thermofisher) read at 450 nm. Assays were done five times.

2.6. NO, PGE2, TNF-α, IL-6 measurements
Assessment of cell culture medium NO levels was done by the Griess reagent while cell culture supernatant collagen II, TNF-α, PGE2, IL-6, MMP13, aggrecan, and ADAMTS5 levels were assessed using ELISA kits (R&D Systems, Minneapolis, MN, United States). All assays were conducted five times.

2.7. Real-time PCR

Extraction of total RNA from chondrocytes that had been exposed to 10 ng/ml IL-1β and gigantol was done using the TRizol reagent (Invitrogen). Total RNA (1 µg) was utilized in cDNA synthesis (MBI Fermentas, Germany). Then, 10 µl of the reaction volume (0.25 µl of each primer, 2 × SYBR Master Mix (5 µl), and dilute cDNA (4.5 µl)) was used for quantitative real-time PCR (qPCR) analyses on a CFX96 Real-Time PCR System (Bio-Rad Laboratories, CA, United States). PCR parameters were: 10 min at 95°C, 40 cycles for 15 s at 95°C and 1 min at 60°C. The obtained cycle threshold (Ct) levels of target mRNA were normalized to GAPDH values. Determination of relative mRNA expression levels for every target gene was done by the 2-ΔΔCt method. The NCBI Primer-Blast Tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used for designing IL-6, iNOS, COX-2, and TNF-α primers, whose sequences were: COX-2 (F) 5′-GAGAGATGTATCCTCCCACAGTCA-3′, (R) 5′-GACCAGGCACCAGACCAAAG-3′; IL-6, (F) 5′-GACAGCCACTCACCCTTTCA-3′, (R) 5′-TTCACCAGGCAAGTCTCCTC-3′; iNOS (F) 5′-CCTTACGAGGGCAGGAAGCAG-3′, (R) 5′-CAGTTTGAGAGAGGAGGCTCCG-3′; TNF-α (F) 5′-GTCAGATCATCTTCTCGA-3′, (R) 5′-CAATGATGGGCTCATACC-3′.

2.8. Western blot analysis

The RIPA lysis buffer and Phenylmethanesulfonyl fluoride (PMSF; 1 mM) were used for proteins extraction from chondrocytes. For 10 min, lysates were placed on ice, centrifuged for 15 min at 12000 rpm, 4°C, after which protein levels were assessed by the BCA protein assay kit (Beyotime). Then, proteins separation was done by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, 40 ng of it transferred to polyvinylidene difluoride membranes (PVDF), blocked at room temperature (RT) for 2 h using non-fat milk (5%), and incubated overnight in the presence of primary antibodies for COX-2 (1:1000), IκB-α (1:1000), iNOS (1:1000), PI3K (1:1000), p65(1:1000), P-PI3K (1:1000), AKT (1:1000), Lamin-B (1:5000), GADPH (1:5000) and P-AKT (1:1000) at 4°C. This was followed by 2 h of incubation at RT with respective secondary antibodies. They were washed 3 times using TBST. Blot visualization was conducted using the electrochemiluminescence plus reagent (Invitrogen). The quantification of blot intensities was done using the image Lab 3.0 software (Bio-Rad).

2.9. Immunofluorescence

To stain collagen II in vitro, cells were seeded in six-well plates, treated with either IL-1β (10 ng/ml) alone or with gigantol (40 µM) for 24 h followed by overnight incubation in serum-free medium. To stain p65, cells were exposed to gigantol and IL-1β for 2 h, rinsed thrice using PBS, fixed in paraformaldehyde (4%), and permeabilized using PBS-dissolved Triton X-100 (0.1%) for 15 min. Cell blocking was done at 37°C for 1 h using bovine serum albumin (5%), rinsed using PBS followed by incubation in the presence of primary antibodies for p65 (1:200) and collagen II (1:200) at 4°C overnight. Glass plates were washed after which chondrocytes were incubated for 1 h in the presence of secondary antibodies (1:400) at RT.
and DAPI-stained for 5 min. Samples were evaluated by fluorescence microscopy (Olympus Inc., Tokyo, Japan). Assessment of fluorescence intensities was done using the Image J software 2.1 (Bethesda, MD, United States).

### 2.10. X-ray imaging method

At 8 weeks post-surgery, mice underwent X-ray imaging to evaluate osteophyte formation, joint space as well as alterations in cartilage surface calcification. Imaging was done by a digitized X-ray machine (Kubtec Model XPERT.8; KUB Technologies Inc) whose settings were: 160µA and 50Kv.

### 2.11. Histopathologic analysis

Fast Green/Safranin-O staining was performed for slides for each joint. Morphologic changes in the chondrocytes as well as in surrounding tissues were microscopically observed and imaged. The OARSI scoring system was used to score medial femoral condyle as well as medial tibial plateau to assess destruction for the articular cartilage. Each group had a total of 15 mice.

Sections were deparaffinized, rehydrated and incubated overnight in the presence of primary antibodies for p-AKT (1: 200) and p-PI3K (1: 200) at 4°C. Sections were washed, incubated for 2 h at RT with secondary antibodies. After hematoxylin and DAB (Zsbio, China) staining, positive cells showed a brownish-yellow color gradient in the nucleus or cytoplasm. The abundance (percentage) of cells that were positively stained cells in the articular surface was determined. Histological assessment was done using 15 mice per group.

### 2.13. Statistical analysis

Data are shown as mean ± S.D. SPSS ver. 20.0 was used for statistical analyses. Comparisons of means among groups was conducted by one-way ANOVA followed by the Tukey’s post hoc test. Comparisons of non-parametric data were done by Kruskal–Wallis H test. Significance was determined at p ≤ 0.05.

### 3. Results

#### 3.1. Effects of gigantol on mouse chondrocyte viability

Figure 1A shows the chemical structure of gigantol. Assessment of gigantol cytotoxicity on chondrocytes was done by the CCK-8 assay using varying concentrations (0,10,20,40,60,100 µM) for 24 and 48 h. Figure 1B and C shows that cell viabilities of mouse chondrocytes got meaningfully immunity from toxic effects of gigantol at 40 µM.

#### 3.2. Effects of gigantol IL-1β-induced inflammatory factor expressions in chondrocytes

Figure 2A, C and D show that gigantol dose dependently (10, 20 and 40 µM) inhibited IL-1β-induced mRNA as well as protein levels of iNOS and COX-2. Nonetheless, differences between the 10µM gigantol
treated group and the IL-1β treatment group were insignificant. In addition, IL-1β treatment elevated endogenous PGE2 and NO levels. Treatment effects of gigantol dose dependently reduced PGE2 expression levels and NO generation (Fig. 2E) and arrived statistical significance expect for the concentration of 10 µM of gigantol. Moreover, qRT-PCR and ELISA results showed that gigantol dose dependently suppressed TNF-α as well as IL-6 levels after IL-1β stimulation, although there was no significant difference at 10 µM. According to the above analysis, the results illustrated the treatment of gigantol attenuated IL-1β-induced inflammation mediators at mRNA as well as protein levels, especially at the concentration of 20 and 40 µM (P < 0.05).

3.3. Effects of gigantol on degradation of the ECM in IL-1β-treated mouse chondrocytes

We investigated gigantol treatment outcomes on collagen-II, aggrecan degradation as well as MMP13 and ADAMTS5 levels in IL-1β treated mouse OA cells. Through ELISA analysis, we found that gigantol not only alleviates IL-1β-mediated inhibition of aggrecan and collagen-II, but dose dependently reversed the promotion effects of IL-1β on expressions of MMP-13 and ADAMTS-5, with statistical significance at 10 and 30 µM (Fig. 3A). Besides, the western blot result also proved that gigantol suppressed collagen-II as well as aggrecan degradation, and reduced ADAMTS-5 as well as MMP-13 protein levels (Fig. 3B and C). Meanwhile, as showed in Fig. 3D and E, the outcomes of immunofluorescence indicated that gigantol markedly reduced collagen-II degradation, consistent with ELISA and western blot results. In short, gigantol attenuated the ECM degradation caused by IL-1β stimulation of mouse OA chondrocytes.

3.4. Effects of gigantol on IκBα degradation and translocation of p65 in mouse OA chondrocytes

As mentioned above, NF-κB signaling pathways play a key function in inflammatory mediator secretion. For further studying the anti-inflammatory effects of gigantol, western blot was performed to assess IκBα protein levels in the cytoplasms of OA cells and p65 protein expressions in the nucleus. IL-1β markedly facilitated the attenuation of IκBα and initiated p65 transfer to the nucleus. However, as described in Fig. 4A and B, gigantol dose dependently restrained these outcomes. Moreover, p65 translocations from the cytoplasm to nucleus were assessed by performing immunofluorescence staining on p65. Interestingly, the p65 active protein of the control group was mainly localized in chondrocyte cytoplasm. Nevertheless, after IL-1β treatment, active p65 protein undergoes a significant cytoplasm to nuclear translocations. As intuitively showed in Fig. 4C, gigantol effectively countered the IL-1β-mediated translocation of p65. In summary, gigantol could inhibit NF-κB pathway activation to protect mouse OA chondrocytes.

3.5. Effects of gigantol on PI3K/Akt signaling pathway activation
As widely reported, PI3K has a vital function in Akt activation and in IL-1β-mediated inflammatory responses. Consequently, PI3K and Akt phosphorylation levels were determined by western blot to intensively establish gigantol effects on the PI3K/Akt axis. Figure 5A and B shows that PI3K as well as Akt phosphorylation were dose dependently inhibited by gigantol pretreatment (10, and 40 µM), and these results had statistical significance (P < 0.01). In summary, the above data and analysis clarify that gigantol dose-dependently suppressed PI3K/Akt signal pathway activation.

3.6. Effects of gigantol on OA development in DMM mouse models

Then, we assessed the protective effects of gigantol in OA occurrence and development. Relative to the sham operation group, joint space in the DMM group was severely narrowed and the cartilage surface density increased. Although the joint space was reduced in the gigantol treated group, cartilage surface calcification was lighter, and the joint space was reduced to a lower degree (Fig. 6A). In addition, histological evaluation of OA by safranin O staining of cartilage revealed that the DMM group showed destruction of the cartilage surface, noticeable hypocellularity as well as substantial proteoglycan loss relative to sham treated group, while gigantol treatment group resulted in more complete and smoother cartilage surface relative to the DMM group (Fig. 6B). Moreover, Fig. 6C shows that the DMM group acquired highest OARSI scores, relative to the sham and gigantol treatment groups, which suggested gigantol treatment partial reversed the cartilage destruction.

Immunohistochemical staining for p-AKT as well as p-PI3K was performed to investigate gigantol effects on activation of PI3K/AKT (Fig. 6D). Few p-AKT and p-PI3K positive areas were detected in the sham group. Contrastingly, the abundance of p-AKT and p-PI3K-positive chondrocytes was high in the DMM group. However, gigantol treatment reversed these effects. These findings were consistent with macrographic findings (Fig. 6E).

4. Discussion

OA, as an irreversible joint degenerative disease, will eventually lead to joint disability, and cause life burdens and huge economic losses to people all over the world. Unfortunately, there are currently no drugs available for osteoarthritis that can stop the progression of the disease and reverse damage. The main goal of current common OA drugs including Nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, topical corticosteroids and tonics is to control pain and improve joint function. However, there is actually a lack of targeted drugs to prevent cartilage damage, which ultimately requires surgical intervention. Therefore, it is important to develop a safe, effective drug for OA. Gigantol, a biphenolic compound found in Dendrobium species, has been reported exhibit numerous biological functions including antitumor effects in human liver and lung cancer, anti-inflammatory effects, and anti-oxidant effects. Nevertheless, as far as we know, there is currently no research exploring the therapeutic effect of gigantol on OA chondrocytes dysfunction. In the present study, we demonstrated the therapeutic
effect of gigantol on IL-1β-induced chondrocytes and OA mice model. Further studies have shown that gigantol exerted the anti-inflammatory mechanism by inhibiting the PI3K/Akt/NF-κB signaling pathway.

As confirmed by previous studies, IL-1β is a principal instigator of OA that stimulates joint tissue to produce several proteases and inflammatory mediators such as MMPs, ADAMTS, NO and PGE2 in cartilage degradation.\textsuperscript{36,37} Among MMPs, MMP-13 belongs to a proteolytic enzyme family and is important in type II collagen and proteoglycan degradation in ECM.\textsuperscript{38,39} Meanwhile, in the pathogenesis of OA, ADAMTS5 has been proved to be closely related to the in the division of aggrecan.\textsuperscript{39} As important inflammatory factors, NO and PGE2 are respectively secreted by INOS and COX-2. It has been reported that promotes MMP secretion and inhibits type II collagen as well as proteoglycan synthesis, leading to degradation of the ECM.\textsuperscript{40} PGE2 promotes ECM degradation by stimulating MMPs and ADAMTS expressions.\textsuperscript{41,42} In this study, gigantol suppressed NO, IL-6, PGE2, and TNF-α levels, which may have had an effect on mRNA as well as protein concentrations of COX-2 and INOS. Furthermore, gigantol suppressed ADAMTS5 and MMPs levels as well as type II collagen degradation and aggregation in mouse OA chondrocytes. Therefore, gigantol downregulated the levels of IL-1β-activated inflammatory cytokines and proteases, and showed the protective effect of ECM.

For all we know, the function of IL-1β in OA is inseparable from NF-κB signaling pathway activation.\textsuperscript{13,14} The NF-κB signaling pathway is a vital catabolic signaling pathway during OA pathogenesis as it regulates inflammatory mediators.\textsuperscript{43} As mentioned above, NF-κB keeps in a resting state by combining with inhibitory protein IkBα in cytoplasm.\textsuperscript{16} Under the initiation by various stimuli including IL-1β, IKK phosphorylates and degrades IkBα.\textsuperscript{44} After losing the binding of IkBα, p65 immediately transfers to the nucleus, involves in gene transcription and ultimately affects the secretion of catabolic enzymes, cytokines as well as inflammatory factors.\textsuperscript{18} The PI3K/Akt signaling pathway is an upstream pathway involved in NF-κB activation.\textsuperscript{45} According to published evidences, after receiving stimulation from cell receptors, PI3K transmits signals through phosphorylating AKT. Phosphorylation of AKT can phosphorylate IkBα and ubiquitinate, which leads to NF-κB activation and transfer to the nucleus.\textsuperscript{46} Therefore, PI3K/AKT pathway suppression can attenuate activation, thereby reducing inflammatory factor secretion and improving ECM destruction.

We confirmed that IL-1β induces PI3K/Akt/ NF-κB pathway activation while gigantol treatment could inhibit the induction. The result was in accord with the former work which suggesting that gigantol attenuate the proliferation of human liver cancer via PI3K/Akt/NF-κB pathway suppression.\textsuperscript{29} In summary, as shown in Fig. 7, our results show that gigantol reduces IL-1β-mediated inflammation in OA cells by downregulating PI3K/Akt/NF-κB activation.

DMM mice models are widely used due to their reliability and effectiveness for the OA.\textsuperscript{47} In our study, cartilage erosion, calcification, chondrocyte loss and ECM degradation in the DMM group were more severe than in sham operation group. These detrimental effects were relieved by gigantol treatment. Therefore, gigantol treatment can alleviate OA progression.
5. Conclusion

Gigantol significantly suppressed IL-1β-induced PI3K/Akt/NF-κB pathway activation to mitigated ECM degradation and inflammatory response in mouse OA chondrocytes. Moreover, treatment of gigantol can play a protective role of OA in DMM-induced OA model. To sum up, the above results indicate that gigantol is likely to become an effective therapeutic agent against OA.

Declarations

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Author Contribution

GZ, KM and MD wrote the manuscript text. GZ, KM and JC prepared figures and collected samples, SZ, KM and HZ analyzed data. HC, and LC designed the experiment and revised the manuscript. All authors contributed to the article and approved the submitted version.

Ethics Statement

All surgical interventions, treatments and postoperative animal care procedures were strictly performed in accordance with the guidelines for Animal Care and Use outlined by the Committee of Wenzhou Medical University.

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Figures

**Figure 1**

Effects of gigantol on the cell viability of chondrocytes. (A) Chemical structure of gigantol. (B and C) The cytotoxic effects of gigantol on chondrocytes were determined at various concentrations for 24 and 48 hours using a CCK8 assay. The values presented are the means ± S.D. of five independent experiments. *P < 0.05, **P < 0.01 vs. the control group, n = 5.
Figure 2

Effects of gigantol on IL-1β-induced inflammation in mouse OA chondrocytes. (A and B) The mRNA expressions of iNOS, COX-2, TNF-α and IL-6 were measured by real-time PCR. (C and D) The protein expressions of iNOS and COX-2 in mouse OA chondrocytes treated as above were visualized by western blot. (E and F) Effects of UA on IL-1β-induced PGE2, NO, TNF-α and IL-6 production in mouse OA chondrocytes. The data in the figures represent the averages ± S.D. Significant differences between different groups are indicated as ###P < 0.01 vs. the control group and *P < 0.05, **P < 0.01 vs. the IL-1β alone treatment group, n = 5.
Figure 3

Effect of gigantol on IL-1β-induced extracellular matrix degradation in mouse OA chondrocytes. (A) The protein expression of Col II, aggrecan, MMP-13, and ADAMTS-5 in culture medium from chondrocytes treated. (B and C) The protein expressions of collagen II, aggrecan, MMP13 and ADAMTS5 in mouse OA chondrocytes treated as above were visualized by western blot. Typical collagen-II (D) was detected by immunofluorescence combined with DAPI staining for nuclei (scale bar: 50 μm). The fluorescence intensities of collagen-II (E) were determined using Image J software. Values represent the averages ± S.D. Significant differences between different groups are indicated as ##P < 0.01 vs. the control group and *P < 0.05, **P < 0.01 vs. the IL-1β alone treatment group, n = 5.
Figure 4

Effects of gigantol on IL-1β-induced NF-κB activation. The protein expressions of IκBα in the cytoplasm and p65 in the nucleus in chondrocytes treated as above were visualized by western blot (A) and are quantified in (B). (C) The nuclear translocation of p65 was detected by immunofluorescence combined with DAPI staining for nuclei (scale bar: 10 μm). The data in the figures represent the averages ± S.D. Significant differences between different groups are indicated as ##P < 0.01 vs. the control group and *P < 0.05, **P < 0.01 vs. the IL-1β alone treatment group, n = 5.
Figure 5

Effect of gigantol on IL-1β-induced PI3K/Akt activation in mouse OA chondrocytes. (A and B) The protein expression levels of P-PI3K, PI3K, P-AKT, AKT and GADPH were determined by western blot and quantification analysis. Data are expressed as mean ± SD. Significant differences among different groups are indicated as ###P < 0.01, vs. control group; *P < 0.05, **P < 0.01, vs. IL-1β alone treatment group, n = 5.
Figure 6

Effects of gigantol on OA development in a mouse DMM model in vivo and digital X-ray images of mouse knee joints. (A) Digital X-ray image of mouse knee joints from different experimental groups. Narrowing of joint space was found in both OA and treatment group, and the calcification of cartilage surface was obviously shown in OA group. (B) Typical safranin O staining of the cartilage and synovitis from different experimental groups at 8 weeks post-surgery (original magnification: ×50 or ×200 and scale bar: 200 μm or 50 μm). (C) Diagrams showing the cartilage OARIS scores. (D) Immunohistochemical staining of p-PI3K, p-AKT expression in the cartilage samples (scale bar: 50 μm). (E) The percentages of p-PI3K, p-AKT
positive cells in each section were quantified by Image Pro Plus. The data in the figures represent the averages ± S.D. Significant differences between different groups are indicated as ###P < 0.01 vs. the sham group and **P < 0.01 vs. the DMM group, n = 15.

Figure 7

Schematic illustration of the potential protective effects of gigantol in osteoarthritis development. Red arrows indicate the inhibiting effects. Green arrows indicate the promoting effects.