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Original article

Gastrodin reduces IL-1β-induced apoptosis, inflammation, and matrix catabolism in osteoarthritis chondrocytes and attenuates rat cartilage degeneration *in vivo*

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ABSTRACT

Therapeutics for osteoarthritis (OA) are intended to restore chondrocyte function and inhibit cell apoptosis. Previous studies have shown that gastrodin had anti-apoptotic and anti- inflammatory effects. However, little is known about whether gastrodin has protective effects against the processes of OA. We studied the potential effects of gastrodin on chondrocytes and the underlying mechanisms. Our results showed that gastrodin could prevent chondrocyte apoptosis induced by IL-1β. Additionally, gastrodin suppressed the nuclear factor kappa B (NF-κB) pathway, decreased the release of inflammatory mediators (IL-6, TNF-α), and reduced matrix catabolism in IL-1β-treated chondrocytes. Furthermore, gastrodin ameliorated rat cartilage degeneration in an OA model of knee joints *in vivo*, suggesting its potential as a candidate therapeutic for OA.

1. Introduction

Osteoarthritis (OA) is the most prevalent chronic degenerative joint disease, affecting tens of millions of people around the world [1]. OA with progressive pain results in severe physical and walking disabilities, which subsequently increase the risk of all-cause mortality [2]. Age, mechanical stress, and inherited factors are considered risk factors contributing to the initiation and progression of OA [3,4]. Further understanding of the relevant molecular mechanism is essential to explore new therapeutic strategies during the process of OA.

Chondrocytes, as the only cell type in articular cartilage, are responsible for OA disease processes. The chondrocytes in articular cartilage keep extracellular matrix (ECM)'s balance between synthesis and degradation, including type II collagen and proteoglycans [5,6]. Pathological features of cartilage degeneration include cell death and associated dysfunction of chondrocytes [7]. Increasing evidence supports that excessive ECM degradation and apoptosis of chondrocytes in OA lead to biological and functional degeneration [8]. However, the exact aetiology of OA remains unclear. The pathogenesis of OA is multifactorial and regulated by a variety of environmental stresses that are related to several signalling pathways, contributing to the progression of OA. Recent studies have reported that increasing pro-inflammation factors, such as interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α) and IL-6, have been found in OA cartilage, which are considered important factors in the development of OA [9]. Increasing evidence has demonstrated that IL-1β, one of the important pro-inflammatory cytokines, enhances catabolic metabolism of by up-regulating metalloproteinases (MMPs), which can accelerate the anabolic activities of chondrocytes via ECM degradation [10,11]. Besides, chondrocytes obtained from patients with OA actively produce nitric oxide (NO), prostaglandins, IL-1β, TNF-α, IL-6, and IL-8. It is demonstrated that the excessive production of cytokines and growth factors by the activated chondrocytes play an important role in the pathophysiology of OA [12–15]. Moreover, pro-inflammatory factors, like IL-1β and TNF-α, have been demonstrated to cause mitochondrial dysfunction and associated apoptosis in chondrocytes [16–18]. IL-1 family is a group of cytokines (11 members), which is a key regulator in immune and inflammatory responses to infections or aseptic injury [6,7]. The proinflammatory IL-1β is the most important cytokine, which has been shown to be directly involved in production of multiple

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proinflammatory mediators such as TNF-α, IL-6 and MMPs, breaking the balance of ECM metabolism and impair its turnover in chondrocytes [8,9]. There was a low expression of IL-1β in normal human serum (0.316 pg/mL), while there is local production of IL-1β in OA cartilage, inducing further downstream mediators, then exerting regulation in multiple pathological processes of OA [1]. Several convincing lines of evidence have shown that inflammation-related changes in OA cartilage are due to activation of NF-κB signalling in chondrocytes [19,20]. Thus, inhibition of these inflammatory mediators or disruption of the inflammatory pathways, and reduced inflammation-associated apoptosis may be effective in the treatment of OA.

Gastrodin, a major active ingredient of Gastrodia elata Blume, has been reported to exert anti-inflammatory and anti-apoptotic effects in various diseases [21,22]. A previous study showed that gastrodin attenuated cerebral ischemic injury via inhibiting inflammatory responses and apoptosis in rats [23]. Furthermore, it has been reported that gastrodin attenuates LPS-induced acute lung injury, via reducing expression of pro-inflammatory factors, such as IL-1β, IL-6, and TNF-α [22].

However, there has been no report on the role of gastrodin in OA. In present work, we sought to examine the effects of gastrodin in OA as well as its working mechanism. We hypothesised that gastrodin may reduce chondrocyte apoptosis and ECM degradation by controlling inflammation. We also assessed the effects of gastrodin on cartilage degeneration *in vivo*. And this new work indicates that gastrodin may have the potential to be a novel drug for the treatment of OA.

2. Materials and methods

2.1. Ethics statement

All the operational procedures and postoperative animal care were in strict accordance with the Animal Care and Use Committee of Wenzhou Medical University (No. 2014-25).

2.2. Reagents

Gastrodin (purity > 98%, structure presented in Fig. 1) and IL-1β were from Sigma (St. Louis, MO, USA). Antibodies against, Bax, Bcl-2, and collagen II were from Abcam (Cambridge, MA, USA). Antibodies against p65, cleaved caspase-3, IκBα, and p-IκBα were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against IL-6, TNF-α, and MMP3 were obtained from Santa Cruz (CA, USA). Other reagents were from Sigma unless otherwise specified.

2.3. Chondrocyte isolation and culture

Chondrocytes were extracted from the bilateral knee joints of SD rats (Animal Center of the Chinese Academy of Sciences, Shanghai, China.). All rats were raised under specific pathogen-free (SPF) standard conditions. The cartilage from knee joints was sectioned into pieces and incubated with 0.25% trypsin/0.02% EDTA for 90 min and then 0.2% type II collagenase at 37 °C in an incubator overnight. After resuspension and filtration, cells were cultured in F12/DMEM medium, including 10% FBS and antibiotics (1% penicillin and streptomycin) in 5% incubator. During passaging, no significant change in cell morphology between primary cells and later passage cells (passage 0–2) was observed. Chondrocytes between passages 0 and 2 were used for experiments except for senescence-associated experiments.

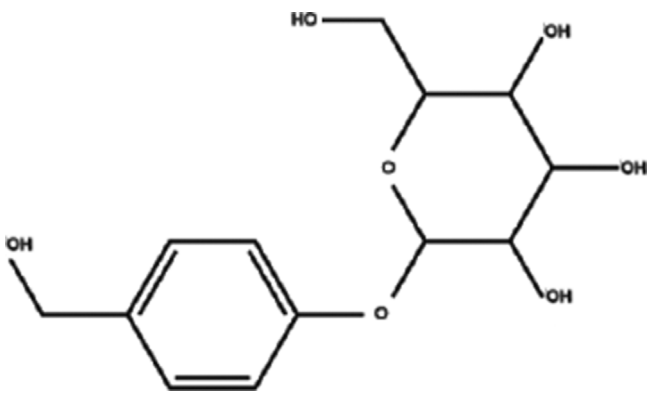

Fig. 1. Chemical structure of gastrodin.

2.4. Cell viability and drug treatment

Chondrocytes were cultured in 96-well plates (5–6 × 10^3 cells/well). After reaching a confluence of 80–90%, the chondrocytes were treated with or without gastrodin with different concentrations (0.1, 1,5, 10, 50, and 100 μM), and stimulated with or without IL-1β (10 ng/mL) for 24 h or 48 h. Then Cell Counting Kit-8 reagent was added in every well according to the introduction. The absorbance was tested at 450 nm using a microplate reader. Analyses of chondrocyte shape and number were used to further validate the effects of GAS. Chondrocytes were passaged and plated in 6-well plates (1 × 10^4 cells/mL), and they were stimulated with IL-1β (10 ng/mL) for 24 h, with incubation with varying concentrations of gastrodin for 2 h. Then, image of chondrocytes were captured using a light microscope (Nikon, Japan).

2.5. Western blot

After treatment, chondrocytes in each group were lysed using RIPA buffer with phosphatase and protease inhibitors. The protein concentration from each experimental group was tested by bicinchoninic acid assay and protein (50 mg) was loaded onto 10–12% SDS-PAGE gels and subsequently transferred to PVDF membrane. The membrane was blocked with 5% BSA for 1 h, and incubated with primary antibodies overnight. Then, the membrane was incubated with the secondary antibody for 1 h. Ultimately, the blots were visualised by the ChemiDicTM XRS + Imaging System (Bio-Rad), normalising to the β-actin signal.

2.6. RT-PCR

After exposure to various gastrodin treatments, total RNA of cells was extracted using the TRIzol method. cDNA was synthesised from total RNA by the PrimeScript-RT reagent kit. And the cDNA was amplificated by the PrimeScript-RT reagent kit and SYBR Premix Ex Taq (Sangon). The level of target genes of each gene was tested using the DDCt method, as described previously [24].

2.7. Immunofluorescence staining

Chondrocytes were passaged on glass cover slips in 6-well plates. After treatment, chondrocytes in each group were fixed by 4% paraformaldehyde (PFA) for 1 h and permeabilised using 0.1% Triton X-100 for 10 min. Following blocking by 5% BSA for 30 min, chondrocytes were incubated with primary antibody against collagen-II (1:500), MMP-3 (1:200), P65 (1:500), and cleaved caspase-3 (1:500) overnight. Then, chondrocytes were incubated with Alexa Fluor 488-conjugated anti-IgGs and Alexa Fluor 647-conjugated anti-IgG secondary antibodies for 1 h and incubated with DAPI for 8 min. Finally, images were observed using a Nikon ECLIPSE Ti microscope (Nikon, Japan).

2.8. TUNEL method

The terminal deoxynucleotidyltransferase (TdT) dUTP nick end-labelling (TUNEL) method was used to measure the apoptotic level of chondrocytes in each group. After fixation with 4% PFA for 1 h, chondrocytes were incubated with 3% H_2O_2 for 10 min and 0.1-0.5%