Gypenoside inhibits interleukin-1β-induced inflammatory response in human osteoarthritis chondrocytes

Zhi-Hong Wan  |  Qing Zhao

Department of Rheumatology and Immunology, Huaihe Hospital of Henan University, Kaifeng 475000, Henan Province, People’s Republic of China

Correspondence
Zhi-Hong Wan
(Email: zhihong_wan@126.com)

Abstract
Gypenoside (GP), the main active ingredient of Gynostemma pentaphyllum, possesses a variety of pharmacological capacities including anti-inflammation, anti-oxidation, and anti-tumor. However, the effects of GP on IL-1β-stimulated human osteoarthritis (OA) chondrocytes are still unknown. Therefore, this study aimed to investigate the anti-inflammatory effects of GP on IL-1β-stimulated human OA chondrocytes and explore the possible mechanism. Our results showed that GP dose-dependently inhibited IL-1β-induced NO and PGE2 production in human OA chondrocytes. In addition, treatment of GP inhibited the expression of MMP3 and MMP13, which was increased by IL-1β. Finally, we found that pretreatment of GP obviously suppressed NF-κB activation in IL-1β-stimulated human OA chondrocytes. Taken together, the results demonstrated that GP has chondro-protective effects, at least in part, through inhibiting the activation of NF-κB signaling pathway in human OA chondrocytes. Thus, these findings suggest that GP may be considered as an alternative therapeutic agent for the management of OA patients.

KEYWORDS
Gypenoside (GP) (OA), inflammation, NF-κB signaling pathway, osteoarthritis

1 | INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease that is characterized by degradation of articular cartilage and joint inflammation. Increasing evidence have reported that inflammatory response plays critical roles in the pathogenesis of OA. Elevated levels of interleukin (IL)-1β lead to the production of matrix metalloproteinases (MMPs) and other inflammatory mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO) in human OA chondrocytes. Current therapeutics such as nonsteroidal anti-inflammatory drugs offer symptomatic relief but disregard disease modification or progression. Therefore, it is imperative to seek safe and effective drugs to treat OA.

Gynostemma pentaphyllum is a traditional Chinese herb medicine that has been used for the treatment of chronic inflammation, hyperlipidemia, and liver disease. Gypenoside (GP), the main active ingredient of G. pentaphyllum, possesses a variety of pharmacological capacities, such as anti-inflammation, anti-oxidation, and anti-tumor. A study by Ye et al. reported that pretreatment with GP significantly suppressed the ischemia/reperfusion (I/R)-induced production of pro-inflammatory cytokines, including IL-1β, IL-6, tumor necrosis factor-α, and interferon-γ in a renal I/R injury mouse model. However, the effects of GP on IL-1β-stimulated human OA chondrocytes are still unknown. Therefore, the goal of this study was to investigate the anti-inflammatory effects of GP on IL-1β-stimulated human OA chondrocytes and explore the possible mechanism. Our results demonstrated that GP attenuated IL-1β-induced inflammatory response in human OA chondrocytes through inhibiting the activation of NF-κB signaling pathway.

2 | MATERIALS AND METHODS

2.1 | Primary human OA chondrocyte culture

OA cartilage tissues were obtained from OA patients [n = 5, mean ± standard deviation (SD) age 65.3 ± 5.7 years] who underwent total knee replacement at the Huaie Hospital (China). In brief, cartilages were minced and digested with 0.25% type II collagenase (Sigma, St. Louis, MO) at 37°C for 6 h. After digestion, the chondrocytes were collected and cultured in DMEM/F12 medium containing 10% FBS and 100 U/mL of penicillin–streptomycin at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Cell passages between one and three were used for experiments. The study was approved by the Ethical Committee of Huaie Hospital and written informed consent was obtained from each participant.

2.2 | Cell viability assay

Cell viability was measured using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) assay according to the manufacturer’s
instructions. In brief, human OA chondrocytes at a density of 1 × 10⁶ cells/well were pretreated with various concentrations of GP (10, 25, 50, or 100 μM) for 2 h prior to IL-1β (5 ng/mL) for 24 h. Then, 10 μL CCK-8 was added to each well and incubated for 4 h. Absorbance at 450 nm was measured with a micro-plate reader (Bio-Rad, Hercules, CA).

### 2.3 | NO and PGE2 measurement

Nitrite levels in culture medium supernatants were measured using spectrophotometric methods utilizing the Griess reagent kit (Sigma). PGE2 levels were detected using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN).

### 2.4 | Western blot

Total protein was extracted from chondrocytes, then washed with ice-cold PBS and lysed with RIPA Cell Lysis Buffer (Takara Biotechnology, Dalian, China) containing a phosphatase inhibitor and the protease inhibitor cocktail (Sigma). Equal amounts of protein (30 μg) were separated by 12% SDS-PAGE and then transferred onto nitrocellulose membranes. The membranes were blocked in 5% skim milk in TBST buffer (5 mM Tris–HCl, pH 7.4, 136 mM NaCl, 0.1% Tween 20) for 1 h at room temperature, and then incubated with 1:1500 dilution of primary antibodies against MMP3, MMP13, p-p65, p65, IκBα, and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) for 2 h. The final quantification of the Western blots was done by Quantity One software (Bio-Rad, Munich, Germany).

### 2.5 | Statistical analysis

All analyses were performed using SPSS version 16.0 software (SPSS Inc., Chicago, IL). The results are presented as the mean ± SD of three independent experiments. Statistical analysis was carried out using one-way analysis of variance followed by Bonferroni test for multiple groups or Student t-test between two groups. P < 0.05 was considered to indicate statistical significance.

### 3 | RESULTS

#### 3.1 | Effects of GP on chondrocyte viability

We first examined the effects of GP on the viability of human OA chondrocytes using the CCK-8 assay. As indicated in Figure 1A, treatment of GP did not affect the viability of chondrocytes at the concentrations of 0–50 μM. However, when the chondrocytes were incubated with 100 μM of GP, the viability of chondrocytes was significantly decreased. Thus, doses ranging from 10 to 50 μM were used in subsequent experiments. Moreover, we found that pretreatment with GP remarkably reversed IL-1β-decreased cell viability (Figure 1B).

#### 3.2 | Treatment of GP inhibited IL-1β-induced NO and PGE2 production in human OA chondrocytes

Overproductions of NO and PGE2 are correlated with the pathophysiology of OA. Therefore, we investigated the effects of GP on the production of NO and PGE2 in IL-1β-stimulated human OA chondrocytes. As shown in Figures 2A and 2B, stimulation of human chondrocytes with IL-1β significantly increased the production of NO and PGE2 compared to unstimulated control. However, pretreatment with GP remarkably prevented IL-1β-stimulated NO and PGE2 production in human OA chondrocytes.

#### 3.3 | Treatment of GP inhibited IL-1β-induced MMP3 and MMP13 expression in human OA chondrocytes

MMPs play an important role in degrading cartilage. Thus, we investigated the effects of GP on MMPs expression in IL-1β-stimulated human OA chondrocytes using Western blot analysis. The results
FIGURE 2 Treatment of GP inhibited IL-1β-induced NO and PGE2 production in human OA chondrocytes. Human OA chondrocytes at a density of $1 \times 10^4$ cells/well were pretreated with various concentrations of GP (10, 25, and 50 μM) for 2 h prior to IL-1β (5 ng/mL) for 24 h. (A) The nitrite levels in the culture medium were assessed by Griess reaction. (B) The levels of PGE2 were investigated using a commercially available ELISA kit. Data are expressed as mean $\pm$ SD ($n$ = 5). *$P < 0.05$ versus control group, #$P < 0.05$ versus IL-1β group.

FIGURE 3 Treatment of GP inhibited IL-1β-induced MMP3 and MMP13 expression in human OA chondrocytes. Human OA chondrocytes at a density of $1 \times 10^4$ cells/well were pretreated with various concentrations of GP (10, 25, and 50 μM) for 2 h prior to IL-1β (5 ng/mL) for 24 h. (A) The protein levels of MMP3 and MMP13 were evaluated using Western blot. (B) The densitometry data were assessed. Data are expressed as mean $\pm$ SD ($n$ = 4). *$P < 0.05$ versus control group, #$P < 0.05$ versus IL-1β group.

3.4 Treatment of GP inhibited IL-1β-induced NF-κB activation in human OA chondrocytes

In order to explore the molecular mechanisms by which GP inhibited inflammatory mediators in response to IL-1β in human OA chondrocytes, we detected the effects of GP on NF-κB activation in IL-1β-stimulated chondrocytes using Western blot analysis. As shown in Figure 4, IL-1β stimulation significantly induced the phosphorylation of NF-κB p65 and IκBα degradation in chondrocytes, as compared with the control group. However, pretreatment with GP obviously suppressed NF-κB activation in IL-1β-stimulated human OA chondrocytes (Figure 4).

4 DISCUSSION

To the best of our knowledge, this study for the first time showed that GP dose-dependently inhibited IL-1β-induced NO and PGE2 production in human OA chondrocytes. In addition, treatment of GP inhibited the expression of MMP3 and MMP13, which was increased by IL-1β. Finally, we found that pretreatment of GP obviously suppressed NF-κB activation in IL-1β-stimulated human OA chondrocytes.

IL-1β has been shown to induce inflammatory mediators in OA.[12] Thus, IL-1β has been widely used to mimic the microenvironment of OA for in vitro studies.[14–16] For these reasons, we selected IL-1β in this study to investigate the effects of GP on IL-1β-stimulated human OA chondrocytes. The results of current study showed that IL-1β treatment caused a significant decrease in cell viability. However, pretreatment with GP significantly reversed this decrease in a dose-dependent manner.

NO generated by iNOS is an important regulator of inflammation. It was reported that IL-1β significantly induced the production of NO in human OA chondrocytes.[17] PGE2, an inflammatory mediator, mediates cartilage matrix degradation via enhancing MMPs activity and other inflammatory cytokines.[18] In accordance with the results of previous studies, in the present study, stimulation of chondrocytes...
NF-κB signaling pathway is known to be important in the progression of OA. NF-κB is retained in the cytoplasm with the IκB-α inactively, IL-1β leads to IκB-α phosphorylation and degradation, subsequently releasing NF-κB, which then translocates to the nucleus and induced various inflammation-related genes, including iNOS and COX-2. Thus, intervention of NF-κB activation might be an effective way to improve and cure OA. Most recently, one study reported that pre-treatment with GP significantly inhibited IκB-α phosphorylation and NF-κB p65 subunit translocation into nuclei in H9c2 cells induced by oxygen-glucose deprivation–reoxygenation. In line with the results of previous studies, herein, we observed that IL-1β stimulation significantly induced the phosphorylation of NF-κB p65 and IκBα degradation in chondrocytes. However, pretreatment of GP obviously suppressed NF-κB activation in IL-1β-stimulated human OA chondrocytes. These data suggest that GP attenuated IL-1β-induced inflammatory response in human OA chondrocytes through regulating the NF-κB signaling pathway.

In conclusion, the results demonstrated that GP has chondroprotective effects, at least in part, through inhibiting the activation of NF-κB signaling pathway in human OA chondrocytes. Thus, these findings suggest that GP may be considered as an alternative therapeutic agent for the management of patients with OA.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES
