Mangiferin Inhibits IL-1β-Induced Inflammatory Response by Activating PPAR-γ in Human Osteoarthritis Chondrocytes

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Abstract—Inflammation has been reported to play critical roles in the development of osteoarthritis. In the present study, we investigated whether mangiferin (MFN) had anti-inflammatory effects in IL-1β-stimulated human osteoarthritis chondrocytes. The cells were treated with various concentrations of MFN in the presence or absence of IL-1β. The production of MMP-1, MMP-3, PGE2, and NO was measured in this study. The expression of NF-kB and PPAR-γ was detected by western blot analysis. MFN inhibited IL-1β-induced inflammatory mediators PGE2 and NO production. MFN also inhibited IL-1β-induced MMP1 and MMP3 production. IL-1β-induced NF-kB activation was significantly inhibited by MFN. In addition, MFN was found to up-regulate the expression of PPAR-γ in human osteoarthritis chondrocytes. PPAR-γ inhibitor GW9662 significantly reversed the anti-inflammatory effects of MFN. These results suggest that MFN inhibits IL-1β-induced inflammatory response in human osteoarthritis chondrocytes by activating PPAR-γ.

KEY WORDS: mangiferin; osteoarthritis chondrocyte; IL-1β; PPAR-γ.

INTRODUCTION

Osteoarthritis (OA), one of the most common degenerative diseases, is characterized by a slow progressive degeneration of articular cartilage [1]. Inflammatory response has been identified as one of the most important factors that initiates and amplifies the disease [2]. Studies showed that chondrocytes played an important role in the development of OA [3]. Stimulating of human chondrocytes with IL-1β could induce the release of inflammatory mediators PGE2 and NO [4, 5]. Previous studies showed that these inflammatory mediators are regulated by NF-kB, an important transcription factor [6]. A growing body of studies indicated that inhibition of NF-kB activation may attenuate the development of OA [7]. Many herbal products have been known to inhibit NF-kB activation [8]. Therefore, to seek novel active components from natural products may represent a useful strategy to treat OA.

Mangiferin, a glucoside of xanthone existing in phytomedicines and food, has been reported to have anti-inflammatory effects. Previous studies showed that MFN inhibited COX-2 and PGE2 production in rat microglial cells [9]. MFN also inhibited TNF-α-induced NF-kB activation in RAW264.7 cells in vitro [10]. In vivo, MFN has the ability to attenuate LPS and D-galactosamine-induced acute liver injury in mice [11]. MFN also inhibited sepsis-induced acute kidney injury [12] and dextran sulfate sodium-induced colitis in mice [13]. However, the protective effect of EA on osteoarthritis remains unclear. This study made a preliminary experiment to investigate the anti-inflammatory potential of MFN on IL-1β-stimulated human osteoarthritis chondrocytes. Furthermore, we tried to investigate the anti-inflammatory mechanism of MFN on IL-1β-stimulated human osteoarthritis chondrocytes.

MATERIALS AND METHODS

Chemicals and Reagents

Mangiferin was purchased from the National Institutes for Food and Drug Control (Beijing, China).
Recombinant human IL-1β was purchased from R&D systems (Minneapolis, MN, USA). ELISA kits for MMP1, MMP3, and PGE2 were purchased from eBioscience Inc (USA). Griess Reagent was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Antibodies for PPAR-γ, p65, p-p65, IkBα, and p-IkBα were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). All other chemicals were of reagent grade.

Cell Culture
Articular cartilage samples were obtained from 18 patients (age 62 ± 8) undergoing total knee replacement surgery. The experiment was in accordance with the Declaration of Helsinki and Tokyo. Primary chondrocytes were isolated from articular cartilage as described previously [14]. The cells were cultured at 37 °C with 5% CO2. Cells between passages 1 to 3 were used in this study [15, 16].

Cell Viability Assay
The effects of MFN on the viability of chondrocytes were evaluated by CCK-8 assay. In brief, chondrocytes (6 × 10^3/well) were seeded in a 96-well plate and cultured for 12 h. Different concentrations of MFN were added in the cells. Twenty-four hours later, 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, USA).

Measurement of MMP1 and MMP3
Chondrocytes were treated with MFN 1 h before IL-1β (10 ng/ml) stimulation. Twenty-four hours later, the levels of MMP1 and MMP3 in supernatants were measured by ELISA (eBioscience Inc, USA) according to the manufacturer’s instructions.

Measurement of PGE2 and NO
Chondrocytes were treated with MFN 1 h before IL-1β (10 ng/ml) stimulation. Twenty-four hours later, the level of NO in supernatants was measured by the Griess reagent according to the manufacturer’s instructions. The level of PGE2 in supernatants was measured by ELISA kit (eBioscience Inc, USA) according to the manufacturer’s instruction.

PPAR-γ siRNA Transfections
Chondrocytes were seeded into 6-well plates and transfected with PPAR-γ siRNA (100 nM) or control siRNA (100 nM) using Lipofectamine 2000 according to the manufacturer’s instructions. Twenty-four hours later, the cells were treated with MFN 1 h before IL-1β stimulation. Finally, the production of MMP1, MMP3, NO, and PGE2 was detected.

Western Blot Analysis
Chondrocytes were lysed in lysis buffer (Beyotime, China) supplemented with complete protease inhibitor cocktail (Roche, IN, USA) for 30 min on ice. A BCA protein assay kit was used to determine the protein concentrations. The proteins (30 μg) were separated by 10% SDS-PAGE and then transferred onto nitrocellulose membranes. After blocking in 5% BSA for 1 h, the membranes were probed overnight with primary antibodies. Then, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 2 h. Finally, the membranes were detected by the enhanced chemiluminescence detection reagents.

Statistical Analysis
All data were presented as means ± SEM of three replicates. Comparisons between groups were analyzed using SPSS 17.0 software via ANOVA followed by Dunnett’s test. P < 0.05 was considered to indicate statistical significance.

RESULTS
Effects of MFN on Cell Viability
The effects of MFN on the viability of chondrocytes were evaluated by CCK-8 assay. The results showed that MFN did not affect the viability of chondrocytes at the concentrations of 0–20 μM. The viability of chondrocytes was reduced by MFN at the concentration of 25 μM (Fig. 1). Therefore, we chose MFN (0–20 μM) in the subsequent experiments.

MFN Attenuates IL-1β-Induced NO and PGE2 Production
To investigate the anti-inflammatory effects of MFN, the effects of MFN on IL-1β-induced NO and PGE2 production were detected in this study. The results showed
that as compared to the control group (NO, 21.22 ± 4.92 μM; PGE2, 51.21 ± 16.22 pg/ml), treatment of chondrocytes with IL-1β resulted in significant increases in the production of NO (163.18 ± 14.63 μM) and PGE2 (431.81 ± 67.42 pg/ml). However, the levels of NO (121.06 ± 15.78, 82.44 ± 11.39, and 41.37 ± 7.26 μM) and PGE2 (311.73 ± 52.12, 173.96 ± 40.78, and 98.42 ± 12.21 pg/ml) of IL-1β-treated cells were significantly reduced by MFN (Fig. 2).

**MFN Suppresses IL-1β-Induced MMP-1 and MMP-3 Production**

MMP-1 and MMP-3 have been reported to play an important role in degrading cartilage. In the present study, the effects of MFN on IL-1β-induced MMP-1 and MMP-3 production were measured by ELISA. The results showed that as compared to the control group (MMP-1, 87.51 ± 31.12 ng/ml; MMP-3, 16.1 ± 2.08 ng/ml), treatment of chondrocytes with IL-1β resulted in significant increases in the production of MMP-1 (672.82 ± 75.92 ng/ml) and MMP-3 (73.21 ± 7.52 ng/ml). However, the levels of MMP-1 (469.02 ± 54.24, 283.25 ± 51.16, and 141.41 ± 30.81 ng/ml) and MMP-3 (51.39 ± 7.51, 36.92 ± 5.93, and 21.96 ± 3.28 ng/ml) of IL-1β-treated cells were significantly reduced by MFN (Fig. 3).

**MFN Inhibits IL-1β-Induced NF-kB Activation**

We investigated whether MFN inhibited inflammatory mediators via NF-kB signaling pathway. In the present study, the effects of MFN on IL-1β-induced NF-kB activation were detected by western blot analysis. The results showed that as compared to the control group, treatment of chondrocytes with IL-1β resulted in significant increases in phosphorylation NF-kB p65 and IκBα. Treatment of MFN significantly inhibited IL-1β-induced NF-kB activation (Fig. 4).

**MFN Inhibits IL-1β-Induced Inflammatory Response by Activating PPAR-γ**

In this study, we firstly detected the effects of MFN on PPAR-γ expression. The results showed that treatment of MFN dose-dependently up-regulated the expression of PPAR-γ. We investigated whether the anti-inflammatory effects of MFN were via activating PPAR-γ. PPAR-γ was knockdown by siRNA and the inhibition of PPAR-γ by siRNA was detected by western blot analysis. The results showed that the expression of PPAR-γ was significantly inhibited by siRNA. Furthermore, the inhibitory effects of
MFN on PGE2 and NO production were inhibited when PPAR-γ was knockdown (Figs. 5 and 6).

**DISCUSSION**

In recent years, mangiferin has been reported to have anti-inflammatory effects. In this study, we investigated whether mangiferin has anti-inflammatory in IL-1β-stimulated chondrocytes. The results showed that mangiferin exhibited anti-inflammatory effects by inhibiting inflammatory mediator PGE2 and NO production. Mangiferin inhibited IL-1β-stimulated inflammatory response by activating PPAR-γ.

Inflammation has been known to be closely associated with the pathogenesis of OA. Chondrocytes play an important role in the development of OA. Once stimulated by IL-1β, chondrocytes release a variety of inflammatory mediators, such as NO and PGE2. These inflammatory mediators lead to the tissues injury and MMP production [17]. MMPs could inhibit type II collagen synthesis and induce degradation of ECM in OA articular cartilage. Therefore, inhibition of the production of inflammatory mediators represents an effective therapeutic approach for OA. To investigate the anti-inflammatory effects of MFN, the effects of MFN on IL-1β-induced PGE2, NO, MMP-1, and MMP-3 production were detected in this study. The results of this study showed that MFN suppressed IL-1β-
induced inflammatory mediator production. These results suggested that MFN had anti-inflammatory effects on IL-1β-stimulated chondrocytes.

PPAR-γ is a member of nuclear receptor family that can be activated by various xenobiotics and natural fatty acids [18]. In recent years, studies showed that many activate compounds extracted from herbal medicines also have the ability to activate PPAR-γ and can be acted as PPAR-γ agonist [19, 20]. Activation of PPAR-γ could regulate genes involved in lipid metabolism and also inflammatory response. Studies showed that activating of PPAR-γ could inhibit inflammatory response [21]. In addition, many herbal medicines have been known to exhibited anti-inflammatory effects by activating PPAR-γ [22]. PPAR-γ agonist could inhibit LPS-induced NF-kB activation and inflammatory cytokines production. To investigate the anti-inflammatory mechanism of MFN, the effects of MFN on PPAR-γ activation were detected. Our results showed that MFN increased the expression of PPAR-γ in a dose-dependent manner. IL-1β-induced NF-kB activation was also inhibited by treatment of MFN. The inhibitory effects of MFN on NF-kB activation were inhibited when PPAR-γ was knockdown. These results showed that MFN inhibited IL-1β-induced inflammation in chondrocytes by activating PPAR-γ, which subsequently inhibited IL-1β-induced NF-kB activation.

In conclusion, the results of the present study demonstrated that mangiferin exhibited anti-inflammatory effects in IL-1β-stimulated chondrocytes through inhibition of

![Fig. 5. Effects of MFN on PPAR-γ expression. The values presented are the means ± SEM of three independent experiments, and differences between mean values were assessed by ANOVA followed by Dunnett’s test. *p < 0.05 vs. control group; *p < 0.05, **p < 0.01 vs. IL-1β group.](image)

![Fig. 6. Knockdown PPAR-γ reversed the anti-inflammatory effects of MFN. The values presented are the means ± SEM of three independent experiments. *p < 0.05 vs. control group; *p < 0.05, **p < 0.01 vs. IL-1β group.](image)
inflammatory mediator production. The inhibitory effects were contributed to the activation of PPAR-γ and inhibition of NF-kB signaling pathway. Mangiferin might be a potential agent for the treatment of osteoarthritis.

ACKNOWLEDGMENTS

This research project was supported by the National Natural Science Foundation of China (30500508) and the Natural Science Foundation of Heilongjiang Provinces (D201112).

COMPLIANCE WITH ETHICAL STANDARDS

The study was in accordance with the Declaration of Helsinki and Tokyo.

REFERENCES

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