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Caffeic acid protects against IL-1β-induced inflammatory responses and cartilage degradation in articular chondrocytes

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ABSTRACT

Osteoarthritis (OA) is a common articular disease that features cartilage loss and destruction. It has been confirmed that inflammation plays major roles in the progression of osteoarthritis. Caffeic acid (CA), a key dietary nutrient commonly found in coffee, has shown its anti-inflammatory properties in various inflammation diseases. However, the effects of CA in osteoarthritis remain explored. Here we investigated the effects of CA on IL-1β induced increased expression of inflammatory factors as well as the degradation of Collagen II and aggrecan in rat chondrocytes. CA prevented the cartilage damage induced by IL-1β in vivo organ culture of articular cartilage. Besides, the IL-1β induced increased production of inflammation factors such as iNOS and COX2 could be inhibited by CA. Additionally, CA could also suppress IL-1β induced expression of cartilage matrix catabolic enzymes such as ADAMTS5 and MMPs. Moreover, CA could prevent IL-1β induced degradation of Collagen II and aggrecan in chondrocytes. Furthermore, CA inhibited NF-κB activity and the activation of JNK pathway. This study reveals that CA inhibits IL-1β induced inflammation responses through suppression of NF-κB and MAPK related JNK signaling pathways. These results demonstrate that CA may provide new avenues for osteoarthritis treatment in future.

1. Introduction

Osteoarthritis (OA) is chronic degenerative joint disease in which patient experience clinical symptoms of joint pain and declining physical function [1]. It is a leading cause of disability among the elderly population and about 10% of men and 18% of women over 60 years old are affected by this disease [2]. Degeneration of articular cartilage is the most common cause of osteoarthritis. It is recognized as an irreversible process in the progression of OA [3]. There is no cure for osteoarthritis and treatment is limited to symptomatic relief or joint replacement [4]. Therefore, new treatment strategies are urgently need to delay the progression of OA.

An imbalance between anabolic and catabolic factors within chondrocytes can induce cartilage destruction. Patients with OA exhibit high levels of IL-1β in the joint cartilage as well as synovial tissues [5]. Work over the past year has reinforced the role of IL-1β in the onset and development of OA [6]. It is considered a key inflammatory cytokine involved in pathogenesis of OA. IL-1β has been shown to down-regulate the synthesis of cartilage matrix and inhibit the anabolic activity of chondrocytes [7]. Moreover, IL-1β directly induces the expression of genes encoding catabolic factors in chondrocytes, such as inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase 2(COX2), aggrecanase-2(ADAMTS5) and matrix metalloproteinase (MMP1, MMP3 and MMP13) [8]. This dual effect makes IL-1β appear to be a reasonable target for OA treatment and medicines that can reverse the effects of IL-1β may provide a potential avenue for OA therapy.

Caffeic acid (3, 4-dihydroxy cinnamic acid, CA), a well-known phenolic phytochemical, is widely distributed in fruits, vegetables, wine and especially in coffee [9]. CA reportedly possess diverse biological potential including anti-oxidative [10], anti-inflammatory [11] and anticancer activities [12]. Previous studies indicated that CA was able to inhibit the gene expression of MMPs in lipopolysaccharide-activated human monocytes [13]. It also could inhibit solar ultraviolet radiation induced COX-2 expression in mouse skin [14]. Furthermore, another investigation indicated that intra-articular injections of caffeic acid phenethyl ester significantly decreased cartilage destruction and reduced loss of matrix proteoglycans in an experimental rabbit osteoarthritis model [15]. However, the exact role of CA in OA is still unclearly. In present study, we investigate the protective effects of CA in chondrocytes and its internal mechanism.

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2. Materials and methods

2.1. Antibodies and reagents

Recombinant rat IL-1β (501-RL-010)was obtained from R&D systems (Minneapolis, MN, USA). Antibody against MMP1 (Cat. No. 10371-2-AP) and P65 (Cat. No. 10745-1-AP) were obtained from Proteintech Group (Wuhan, China). Antibodies against aggrecan (Cat. No. ab36861) was purchased from Abcam (Cambridge, UK). Antibodies against Collagen II (Cat. No. sc-28887), iNOS (Cat. No. sc-7271) and MMP13 (Cat. No. sc-30073) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against MMP3 (Cat. No. 14351), COX2 (Cat. No. 12882), P38 (Cat. No.8690), P-P38 (Cat. No. 4511), ERK 1/2 (Cat. No.4695), P-ERK (Cat. No.4370), JNK (Cat. No.9258), P-JNK (Cat. No.9255), P-C-JUN (Cat. No. 9165) and P-P65 (Cat. No. 3033) were supplied by Cell Signaling Technology Inc (Beverly, MA, USA). Antibodies against GAPDH (Cat. No. BM3876), ADAMTS5 (Cat. No.BA3020), FITC-conjugated goat anti-rabbit secondary antibody (Cat. No. BA1105), Cy3- conjugated goat anti-rabbit secondary antibody (Cat. No.BA1032), horseradish peroxidase-linked (HRP) goat anti-rabbit (Cat. No. BA1054) and goat anti-mouse antibodies (Cat. No. BA1050) were purchased from Boster (Wuhan, China). Caffeic acid (Cat. No. C0625) was provided by Sigma Aldrich (St. Louis, MO, USA) and was dissolved indimethyl sulfoxide (DMSO). Dulbecco' s modified Eagle's medium F12 (DMEM /F12) and fetal bovine serum (FBS) were obtained from Gibco (NY, USA).

2.2. Cell culture

Chondrocytes were obtained from Sprague-Dawley rats (5 days-old ; the Animal Care and Use Committee of Tongji Medical College, Wuhan, China). Briefly, rats were firstly sacrificed by cervical dislocation. Next, articular cartilage from the Knees were dissected, minced and digested primarily with 0.25% trypsin for 30 min at 37 °C and subsequently with 0.25% collagenase II for 8 h at 37 °C. Cells were then collected by centrifugation (400× g for 8 min), re-suspended and cultured in DMEM /F12 (1:1) culture medium containing 10% FBS plus 1% penicillin/streptomycin solution at 37 °C with 5% CO2. Two to three passages were used in the next experiments and the animal procedures were approved and under supervision by the Animal Care and Use Committee of Tongji Medical College, Wuhan, China.

2.3. Cell viability assay

Cell viability was analyzed by using a Cell counting Kit-8(CCK8, Boster, and Wuhan, China). Chondrocytes (1×10^4 cells/well) were firstly seeded into 96-well plates. After 24 h of cell adhesion, cells were then treated with various concentrations of CA alone or in combination with IL-1β (10 ng/ml) for 24 h. Following incubation, 10 μL CCK-8 solution dissolved in 100 μl culture medium were added into wells. The plates were then incubated for additional 1 h at 37 °C. Finally, the absorbance of the solution at 450 nm was measured by using a plate reader (Bio-Rad, Richmond, CA, USA).

2.4. Ex vivo analysis by organ culture of rat articular cartilage

The articular cartilage explants were harvested from the knee joint of 3-week-old Sprague-Dawley rats [16]. The explants were initially cultured at 37 °C with 5% CO2 in 10% FBS containing 1% penicillin/streptomycin solution for 2 days. Next, the explants were washed in serum free medium and placed in 48-well plates. Explants were cultured in serum free medium containing 10 ng/ml IL-1β alone or 10 ng/ml IL-1β with 20 μg/mL CA for a further 3 days. Then, explants were collected and fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 24 h. Finally, samples were paraffin-embedded, sectioned at 6 μm and stained with hematoxylin and eosin (H&E).

2.5. Western blotting analysis

Chondrocytes were seeded in a sterile six-well plate and incubated at 37 °C with 5% CO2. When the cell density reaches 80%, the cells were exposed to IL-1β (10 ng/ml) alone or CA (5, 10, or 20 μg/mL) in combination with IL-1β (10 ng/ml) for 24 h. Cells were then lysed in ice-cold radio immunoprecipitation assay (RIPA) buffer containing 1% protease and 1% phosphatase inhibitors (Boster, Wuhan, China). The total protein levels were determined using a bicinchoninic acid (BCA) protein assay kit (Boster, Wuhan, China) and equal amounts of protein were resolved on a 12% polyacrylamide gel. Separated protein was transferred to 0.45-μm polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). The blots were blocked with 5% bovine serum albumin (BSA) in Tris-buffered Saline-Tween solution (TBST) for 1 h at room temperature and then incubated with specific primary antibodies (P-P38, P38, P-ERK, ERK, P-JNK, JNK, P-C-JUN, MMP1, MMP3, COX-2, P-P65 and P65 at 1:1000 dilution; iNOS, MMP13, ADAMTS5 and GAPDH at 1:500 dilution) at 4 ℃ overnight. The membranes were next incubated with the corresponding horseradish peroxidase-linked goat anti-rabbit (1:5000 dilution) or goat anti-mouse antibodies (1:5000 dilution) at 37 °C for 1 h after washing with TBST. Membranes were then visualized with an enhanced chemiluminescence reagent (Boster, Wuhan, China). Relative expression was quantified using the Bio-Rad Image Lab system version 5.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and compared to GAPDH.

2.6. Immunofluorescence

Chondrocytes were seeded at 1×10^4 cells/ well and cultured in a 24 well plate. After reaching confluence, the cells were stimulated with 10 ng/ml IL-1β with or without 20 μg/ml CA. Next, cells were fixed with 4% paraformaldehyde for 15 min at 37 °C. The PBS containing 0.2% Triton X-100 was used to permeabilize the cells for 15 min and 5% BSA was used to block the cells for 30 min. Cells were then incubated with Collagen II (1:100 dilution), aggrecan (1:100 dilution) and P65 (1:100 dilution) for 14 h – 16 h at 4 °C. After washing three times with PBS, cells were next incubated with an FITC-conjugated goat anti-rabbit secondary antibody (1:50 dilution) and Cy3- conjugated goat anti-rabbit (1:50 dilution) secondary antibody at 37 °C for 1 h in the dark. The cells were then washed three times with PBS and incubated with 4–6-Diamidino-2-phenylindole (DAPI) for 10 min. Images were next acquired with a fluorescence microscope (Evos fl auto, life technologies, USA).

2.7. Statistical analysis

Data were presented as the mean ± standard deviation (SD). All of the analysis was performed using Graph Pad Prism software. Differences among treatments were analyzed by one-way ANOVA followed by Tukey post hoc test. Values of $P < 0.05$ were considered statistically significant.

3. Result

3.1. Effects of CA on chondrocyte viability

We first examined whether CA was toxic to chondrocytes. As shown in Fig. 1, the results of the CCK8 assay demonstrated that administration of CA (5, 10, or 20 μg/mL) had no significant effect on cell viability. Besides, we found that IL-1β treatment for 24 h couldn't obviously decrease the cell viability.

3.2. CA suppressed IL-1β induced expression of iNOS and COX2 in chondrocytes

Western blot analysis was performed to detect the protein