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Original Article

Chlorogenic acid suppresses interleukin-1β-induced inflammatory mediators in human chondrocytes

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Abstract: We investigated the anti-inflammatory properties of chlorogenic acid (CGA) in interleukin-1β-induced chondrocytes. The nitric oxide (NO) and prostaglandin E2 (PGE_2) were detected by Griess and Enzyme-linked immunosorbent assay (ELISA) respectively. Quantitative real-time PCR and western blot were performed to measure the expression of inducible NO synthase (iNOS) and cyclooxygenase (COX)-2. Our results indicate that CGA inhibited the production of NO and PGE_2 as well as the expression of iNOS and COX-2 in chondrocytes. Our data suggest that CGA possess potential value in the treatment of OA.

Keywords: Chlorogenic acid, inducible NO synthase, cyclooxygenase, chondrocyte

Introduction

Osteoarthritis (OA) is the most disorder of the joint which is characterized by cartilage damage progressively. It is well known that matrix metalloproteinases (MMPs) is the main mediator in cartilage degradation in OA [1]. However, the importance of inflammation in OA is also recognized [2]. The pro-inflammatory cytokine interleukin-1β (IL-1β) plays a critical role in OA progression. Previous studies show that IL-1β can trigger a cascade of inflammation events in OA [3], it can also induce the expression of MMPs, leading to cartilage matrix degradation [4]. In addition, IL-1β can induce the production of nitric oxide (NO) and prostaglandin E2 (PGE_2), two inflammatory mediators which are associated with cartilage degradation in OA [5].

Nonsteroidal anti-inflammatory drugs (NSAIDs) were wide-spread used in treatment of OA to reduce the pain of joint. These drugs can exert side effect for long-term use, thus, it is necessary to find new agent for alternative treatment in OA.

Chlorogenic acid (CGA) is a type of polyphenol which is rich in many plants. CGA been reported to possess antioxidative, anti-cancer and anti-inflammatory properties in vitro and in vivo [6, 7]. In previous study, we found that CGA reduced the MMPs expression in chondrocytes [8]. However, the effect of CGA on IL-1β-induced inflammatory responses of chondrocytes is still unclear. In the present study, we investigated the effect of CGA on the expression of inflammatory mediators in IL-1β-induced chondrocytes.

Materials and methods

Reagents and antibodies

Recombinant human IL-1β and CGA was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium DMEM penicillin, streptomycin, fetal bovine serum (FBS), 0.25% trypsin, and collagenase II were purchased from Gibco BRL (Grand Island, NY, USA). Monoclonal antibodies for Inducible NO synthase (iNOS), Cyclooxygenase-2 (COX-2), β-actin and peroxidase-conjugated secondary antibody were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Preparation of chondrocytes

This study was approved by the local Ethics Committee. Cartilage was obtained from OA patients undergoing total knee arthroplasty

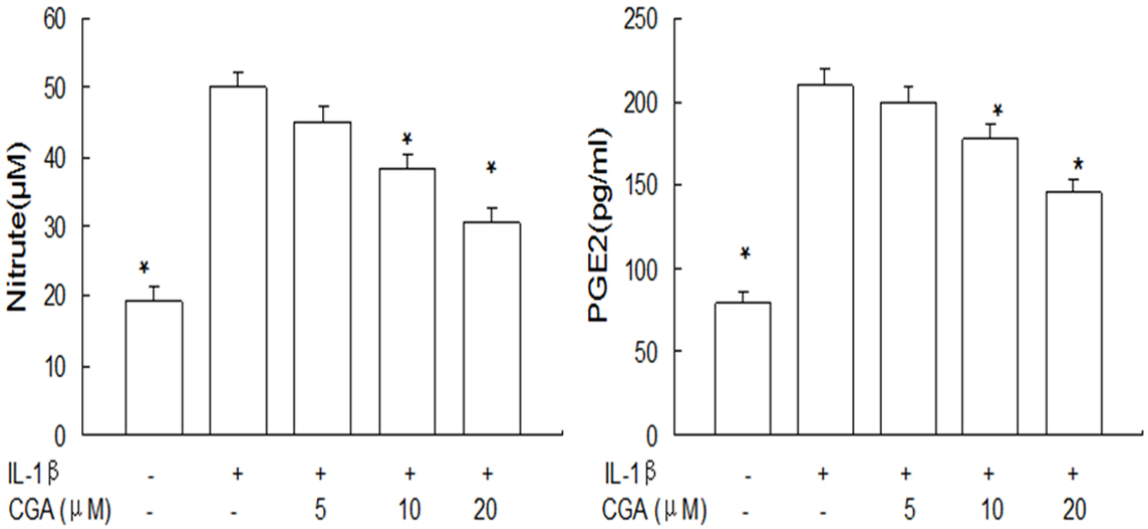

Figure 1. Effects of CGA on NO and PGE_2 production in IL-1β-induced chondrocytes. Cells were pre-treated with 5-20 μM of CGA for 1 h, followed with or without IL-1β (10 ng/ml) for 24 h. NO and PGE_2 production were measured by Griess reaction and ELISA respectively. Data are expressed as mean ± standard deviation (SD). *$P < 0.05$ compared with cells stimulated with IL-1β. The experiment is representative of three experiments performed.

after obtaining informed consents from patients. Chondrocytes were isolated and cultured as previously described [9]. In brief, Cartilage received enzymatic digestion with 0.25% trypsin for 30 min and 2 mg/ml collagenase II for 6 hours. Cells were cultured in DMEM with 10% FBS and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C with 5% CO_2 and 95% air. Cells from passages 3-4 were used in the experiment.

Measurements of PGE_2 and NO production

Chondrocytes were cultured in 6-well plate, cells were serum starved, pre-treated with different concentrations of CGA for 1 h followed by stimulation with IL-1β (10 ng/ml) for 24 h. culture supernatants were collected. For PGE_2 production, ELISA kit was used and Griess reaction was performed for NO production, Cells were collected for real-time polymerase chain reaction (PCR) and western blotting.

Quantitative real-time polymerase chain reaction (PCR)

Quantitative PCR was performed as previously reported [9]. In brief, total RNA was isolated by TRIzol reagents (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed to obtain cDNA. Then, quantitative real-time PCR were performed using an iCycler apparatus system (Bio-Rad). The primer sequences were as follows: PCR as described above. The primers used were as follows: for iNOS: 5'-CTCAGCAGCATCCACGCCAAGAA-3' and 5'-CCTGAGAAGGACGAGTGGACAT-3'; for COX-2: 5'-CAGCAAAGGCTACTTGGATCAGG-3' and 5'-CCTGAGAAGGACGAGTGGACAT-3'; for GAPDH: 5'-CAACGGCACAGTCAAGGCTGAGA-3' and 5'-CTCAGCACCAGCATCACCCCAT-3'; GAPDH was used as an internal control. The real-time PCR data were quantified using the ΔCT method with the formula: $n = 100 \times 2^{-(\Delta CT \text{ targeted gene} - \Delta CT \text{ GAPDH})}$.

Western blotting

Chondrocytes were washed with PBS, collected by scraper and lysed using the cell lysis buffer. Then, the lysates were resolved in SDS-PAGE, transferred to PVDF membranes, probed with primary antibodies for iNOS and COX-2 overnight at 4°C. After washing, membranes were incubated with HRP-linked secondary antibodies for 1 h at room temperature. Membranes were visualized by Enhanced Chemiluminescence (ECL) kit and exposed to X-ray films (Kodak, Hangzhou, China).

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) and were analyzed statistically

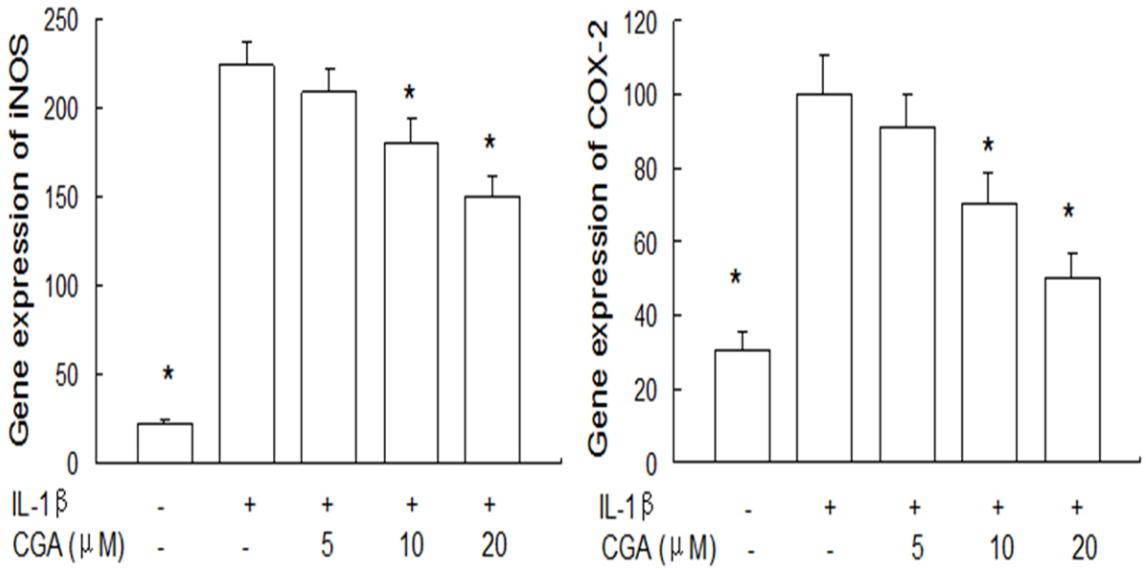

Figure 2. Effects of CGA on iNOS and COX-2 gene expression in IL-1β-induced chondrocytes. Cells were pre-treated with different concentrations of CGA for 1 h prior to IL-1β (10 ng/ml) for 24 h. Gene expression of iNOS and COX-2 was detected by quantitative real-time PCR. Data are expressed as mean ± standard deviation (SD). *$P < 0.05$ compared with cells stimulated with IL-1β. The experiment is representative of three experiments performed.

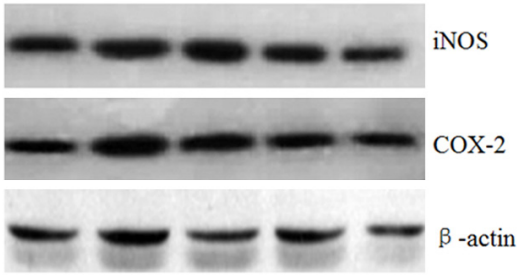

Figure 3. Effects of CGA on IL-1β-induced iNOS and COX-2 protein levels in IL-1β-induced chondrocytes. Cells were pre-treated with different concentrations of CGA for 1 h prior to IL-1β (10 ng/ml) for 24 h. The protein levels of iNOS and COX-2 in chondrocytes were assessed by western blot analysis.

using one-way ANOVA. Differences were considered significant when *P* values less than 0.05.

Results

Effects of CGA on NO and PGE_2 production in chondrocytes

We investigated whether CGA had inhibitory effect on IL-1β-induced NO and PGE_2 production. Our results showed that CGA suppressed IL-1β-induced NO and PGE_2 production (**Figure 1**).

Effects of CGA on iNOS and COX-2 expression in chondrocytes

We next investigate the effect of CGA on iNOS and COX-2 gene expression and protein levels in IL-1β-induced chondrocytes. Our results showed that CGA suppressed the iNOS and COX-2 mRNA expression (**Figure 2**) as well as the protein levels of iNOS and COX-2 (**Figure 3**).

Discussion

Until now, disease modifying anti-OA drugs (DMOADs) are lacking, so there is necessary to find new agent to modify OA. In this field, natural products are considered as a source of new agents. Previous studies have shown that CGA has anti-inflammatory activities and inhibitory effects on MMPs. However, the anti-inflammatory effects of CGA in chondrocytes are still unclear. Therefore, we investigated the anti-inflammatory effect of CGA in IL-1β-stimulated chondrocytes. We demonstrated that CGA not only suppressed the production of NO and PGE_2, but also inhibited iNOS and COX-2 expression in IL-1β-induced chondrocytes.

There is cumulated evidences showed that iNOS-NO signaling pathway are implicated in the pathogenesis of OA [10]. Previous study has shown that IL-1β can induce NO production via

iNOS in chondrocytes [11]. Inhibition of iNOS-NO signaling pathway is beneficial to OA [12]. In the present study, we found that CGA reduced IL-1β-induced iNOS-NO activation in a dose-dependent manner. Our results are partly supported by previous study which reported that CGA reduced NO and iNOS expression *in vitro* [13].

In the present study, we demonstrate that CGA suppressed the PGE_2 via inhibiting COX-2 expression in IL-1β-induced chondrocytes. PGE_2 is an inflammatory mediator involved in the pathogenesis of OA. Because the synthesis of PGE_2 is dependent on COX-2, in the present study, we investigated whether CGA possessed inhibitory effect on PGE_2 and COX-2, our results showed that CGA inhibited the elevated PGE_2 and COX-2 expression in chondrocyte. Our results are consistent with previous studies showing that CGA reduced the COX-2-PGE_2 signaling pathway in other cells [14].

Nuclear factor-kappaB (NF-κB) is a transcription factor that plays an important role in inflammation events including OA. It is known that the effects of IL-1β in OA are associated with NF-κB [15]. In our previous study, we demonstrated that CGA inhibited NF-κB activation in chondrocytes. Thus, we speculated that the anti-inflammatory effects of CGA in IL-1β-induced chondrocytes are partly associated with the inhibition of NF-κB.

In conclusion, our findings showed that CGA exerts an anti-inflammatory effect by the inhibition of COX-2/PGE_2 and iNOS/NO expression and the anti-inflammatory effect may partly associate with the inhibition of NF-κB.

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Disclosure of conflict of interest

None.

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