Chondroprotective effect of curcumin and lecithin complex in human chondrocytes stimulated by IL-1 β via an anti-inflammatory mechanism

Leeseon Kim¹ · Ji Yeon Kim¹

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Abstract A complex of curcumin and lecithin developed to improve the solubility of curcumin, enhanced its chondroprotective effect via an anti-inflammatory mechanism. In macrophage, proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, prostaglandin E2 (PGE2), and nitric oxide (NO) were quantified. In addition, the activity of nuclear factor (NF)- κ B was examined. With chondrocytes, inflammatory mediators were assessed by measuring the secretion levels of IL-6, IL-8, and PGE2, also the mRNA expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Metalloproteinases (MMPs), tissue inhibitor of metalloprotease (TIMP)-1, type II collagen (COL2), proteoglycan (PG), and hyaluronic acid (HA) were measured with respect to the articulation surface. The complex promoted the anti-inflammatory effect by the inhibition of inflammatory mediators. In addition, mRNA expression levels ameliorated. Furthermore, it was effective in decreasing extracellular secretion of polypeptides, also corresponding intracellular MMPs and TIMP-1. In conclusion, the complex may be developed as a functional supplement to maintain articulation health.

Keywords Ant-inflammatory effect · Chondroprotective effect · Curcumin · Lecithin complex · Osteoarthritis

Introduction

Osteoarthritis (OA) is a common chronic illness that affects the elderly worldwide. There is a gradual destruction of cartilage in the joints as well as the synovial membrane, peritoneum, and soft tissue surrounding the joints (Zhang et al., 2008). Currently, management of OA includes a combination of non-medical and pharmacological approaches (Zhang et al., 2008). It is easy to detect the pro-inflammatory cytokines produced by infiltrating inflammatory cells and chondrocytes in synovial fluid of OA patients (Malemud, 2004). Chondrocytes, the only cells in the cartilage joint, serve to balance the synthesis and degradation of extracellular substrates (Wang et al., 2011). In OA, chondrocytes release IL-1 β , which can induce production of metalloproteinases (MMPs) resulting in the degeneration of cartilage (Lianxu et al., 2006). Important mediators of matrix degradation are inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) enzymes, which promote the release of nitric oxide (NO) and prostaglandin E2 (PGE2) (Stadler et al., 1991; StefanovicRacic et al., 1997; Taskiran et al., 1994). These inflammatory mediators cause pain and increase inflammatory responses (Vergnolle et al., 2001).

Curcumin is a well-known food component having anti-inflammatory effects in disorders such as rheumatoid arthritis and inflammatory bowel disease (Bundy et al., 2004; Garcea et al., 2004; Hanai et al., 2006; Holt et al., 2005; Sharma et al., 2004).

Although the effect of curcumin has been demonstrated, it shows low solubility in food matrix and poor bioavailability (Anand et al., 2007; Mahran et al., 2017). The bioavailability of curcumin can be enhanced by formulation with soy lecithin (Semalty et al., 2010). Soy lecithin has affinity for polyphenolics, and breaks down the

✉ Ji Yeon Kim
jiyeonk@seoultech.ac.kr

¹ Department of Food Science and Technology, Seoul National University of Science and Technology, Seoul 01811, Korea

supramolecular state by possessing a clear stoichiometry (Semalty et al., 2010). The lecithin complex had an increased rate and range of solubility in aqueous intestinal fluids and ability to cross biomembranes (Semalty et al., 2010).

A previous study demonstrated that the lecithin complex improved OA symptoms, which was rated by Western Ontario and McMaster Universities (WOMAC) score (Belcaro et al., 2010). Other studies have evaluated the effect of lecithin complex associated with diabetic microangiopathy (Appendino et al., 2011) and its anti-inflammatory properties (Farinacci et al., 2009). However, the anti-inflammatory effect of the curcumin and lecithin complex on chondrocytes has not been studied. Therefore, the purpose of the present study was to examine whether the curcumin and lecithin complex could impart a chondroprotective effect through an anti-inflammatory mechanism in chondrocytes as well as macrophages.

Materials and methods

Chemical and reagents

A complex of curcumin and lecithin (Meriva®) was prepared by mixing oleoresin turmeric extract and soy lecithin and kindly provided by Sam-oh pharm (Gangnam, Seoul, Korea). The curcumin content of the complex was 20%. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin were procured from Biowest (Nuaillé, Cholet, France). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was procured from Gibco (Rockville, MD, USA). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). IL-1 β was obtained from Peprotech (Rocky Hill, NJ, USA).

Cell culture

RAW 264.7 cell line was procured from KCLB (Korea Cell Line Bank, Seoul, Korea). For maintenance, cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and penicillin–streptomycin, HEPES at 37 °C at atmosphere of 5% CO₂/95% air. For the experiments, RAW 264.7 cells were seeded at 1×10^5 cells/well in 6-well cell culture plates. After 24 h, they were treated with the curcumin and lecithin complex (0.5–20 μ M as curcumin) and LPS (1 μ g/mL) for 48 h. Finally, the culture supernatant was isolated to measure NO, PGE2, TNF- α , IL-6, IL-1 β levels. RAW 264.7 cells were assembled for determination of nuclear factor kappa B (NF- κ B) production and mRNA expression levels.

Human chondrocytes (HCH-c cells) were purchased from PromoCell (Sickingenstr, Heidelberg, Germany). For routine maintenance, cells were grown in chondrocyte growth medium with supplement mix from PromoCell at 37 °C in an atmosphere of 5% CO₂/95% air under saturating humidity. For the experiments, HCH-c cells were seeded at 1×10^5 cells/well in 6-well culture plates. After 24 h, they were treated with the curcumin and lecithin complex (0.5–20 μ M as curcumin) and IL-1 β (10 ng/mL) for 48 h. Finally, the culture supernatant was collected to measure MMPs, tissue inhibitor of metalloprotease (TIMP)-1, type II collagen (COL2), proteoglycan (PG), and hyaluronic acid (HA), PGE2, IL-6, IL-8 levels. The HCH-c cells were collected for determination of mRNA levels.

Measurement of NO, PGE2, proinflammatory cytokines, MMPs, TIMP-1, COL2 and PG levels

The cell culture supernatant (100 μ L) was blended with 100 μ L of Griess reagent [2.5% phosphoric acid containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride and 1% sulfanilamide] and incubated at 37 °C. After 10 min, the absorbance was assessed at 540 nm. Nitrite levels were determined using a standard sodium nitrite curve. The cell culture supernatant was also used to detect the levels of PGE2, TNF- α , IL-6, IL-1 β . The levels of PGE2, TNF- α , IL-6, IL-8, IL-1 β , MMPs, TIMP-1, HA, COL2, PG were assessed using enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Rockford, IL, USA) according to the manufacturer's directions. The levels were quantified using a standard curve provided with the kit. The experiment was performed in triplicate.

RNA isolation, cDNA synthesis and quantitative Real-Time PCR

Total RNA was isolated from RAW 264.7 and HCH-c cells using TRIzol. The RNA concentration and quality were measured by BioSpec-nano spectrophotometer (Mecasys Corp., Daejeon, Korea). cDNA was synthesized using a High Capacity RNA-to-cDNA kit (Hoffmann La Roche, Basel, Switzerland). The StepOnePlus Real-Time PCR system (Hoffmann La Roche, Basel, Switzerland) along with the Universal ProbeLibrary (UPL) probe method was used to quantify RNA. Amplifications were performed at 95 °C with a 10 min template denaturation, followed by 40 cycles at 95 °C for 10 s each and 60 °C for 30 s each. The relative amounts of mRNAs were normalized by β -actin and were assessed by comparative Δ CT method. The sense and antisense sequences were shown in Table 1.

Table 1 Primer sequences for RT-PCR

Cell line	Gene name	Oligonucleotide sequence
Raw 264.7	COX-2	F: 5'-GATGCTCTTCCGAGCTGTG-3' R: 5'-GGATTGGAACAGCAAGGATTT-3'
	iNOS	F: 5'-CTTTGCCACGGACGAGAC-3' R: 5'-TCATTGTACTCTGAGGGCTGAC-3'
	β -actin	F: 5'-CTAAGGCCAACCGTGAAAAG-3' R: 5'-ACCAGAGGCATACAGGGACA-3'
	MMP-1	F: 5'-GCTAACCTTTGATGCTATAACTACGA-3' R: 5'-TTTGTGCGCATGTAGAATCTG-3'
	MMP-2	F: 5'-CCCCAAAACGGACAAAGAG-3' R: 5'-CTTCAGCACAAACAGGTTGC-3'
	MMP-3	F: 5'-CAAAACATATTCTTTGTAGAGGACAA-3' R: 5'-TTCAGCTATTTGCTTGGGAAA-3'
HCH-c	MMP-9	F: 5'-GAACCAATCTCACCGACAGG-3' R: 5'-GCCACCCGAGTGTAACCATA-3'
	MMP-13	F: 5'-CCAGTCTCCGAGGAGAAACA-3' R: 5'-AAAAACAGCTCCGCATCAAC-3'
	β -actin	F: 5'-AAGTCCCTTGCCATCCTAAAA-3' R: 5'-ATGCTATCACCTCCCCTGTG-3'

NF- κ B activity in macrophages

Nuclear lysates were measured by the relative binding of p65 nuclear transcript using NF- κ B p50/p65 Transcription Factor Assay kit (ab133128) from Abcam (Cambridge, MA, USA). In short, NF- κ B including a specific double stranded DNA sequence was immobilized onto well plate for analysis of p65 binding which was determined by antibody binding and horseradish peroxidase (HRP) conjugate detection. Absorbance was detected at 450 nm.

Statistical analysis

Results were described as the mean $\pm$ standard deviation (SD). Statistical analyses were performed by Dunnett's test analysis of variance (ANOVA) (SPSS 20, SPSS Inc., Chicago, IL, USA). *P* values < 0.05 were considered to be statistically significant (versus LPS or IL-1 β -treated group).

Results and discussion

Effect of complex of curcumin and lecithin on RAW 264.7 cells

The complex of curcumin and lecithin was developed, in order to improve bioavailability of curcumin (Marczylo et al., 2007). However, although the active compound, curcumin, is preserved in a complex solution, the anti-inflammatory effect of the complex had not been confirmed.

To confirm the anti-inflammatory properties of the complex, the RAW 264.7 cells were treated with the complex to induced inflammation using LPS. Transcription factors in the NF- κ B family regulate various genes to treat inflammation (Chen, 2006). NF- κ B is composed of p50 and p65 subunits and exists in an inactive form in the cytosol associated with the inhibitor protein I κ B α . When NF- κ B is activated, the expression of proinflammatory genes increases (Oh et al., 2001). As curcumin is well known to regulate anti-inflammatory effects, primarily through the deactivation of NF- κ B, (Sandur et al., 2007). This study also confirmed the effects of the complex on LPS-induced NF- κ B activation. The inhibition of complex was observed with 10 and 20 μ M (Fig. 1A) and this concentration was in agreement with previous reports (Ben et al., 2011; Kim, 2011; Pae et al., 2008; Zhao et al., 2010). This study also demonstrated that the complex inhibited the production of TNF- α , IL-6, and IL-1 β stimulated by LPS (Fig. 1B). The complex also suppressed iNOS and COX-2 mRNA expression and inhibited NO and PGE2 production (Fig. 1C and D).

Effect of curcumin and lecithin complex on IL-1 β -induced inflammation of HCH-c cells

In their natural state, chondrocytes show low mitotic trend and retain their function by synthesis of ECM-related proteins such as COL2 (Jackson et al., 2006). In the ECM, PGs combined with water provide joint elasticity, and confer strength (Jackson et al., 2006). PGs developed broad network throughout the ECM (Goggs et al., 2003) and also

Fig. 1 NF-κB activity (A); comparison of TNF-α, IL-1β, IL-6, and PGE2 (B); concentration profile of NO (C) production; relative mRNA expression levels of the genes COX-2 and iNOS (D) in Raw 264.7 cells stimulated with LPS. Cells were seeded at 1.0×10^5 cells/

mL, then stimulated with LPS and sample for 48 h. To calculate the relative mRNA expression levels, the $\Delta\Delta CT$ method was used. The mRNA levels were normalized by β -actin. *Significant different from the LPS values by ANOVA followed by Dunnett's test ($p < 0.05$)

composed with core protein like GAGs or HA (Jackson et al., 2006).

MMPs are degrading and remodeling enzymes that are capable of degrading all kinds of ECM proteins in normal physiological processes; however, in OA, stimulation of MMPs leads to excessive extracellular ECM degradation. (Billinghurst et al., 1997; Goggs et al., 2003). MMP-1 and -13 were known as collagenases, MMP-2 and -9 were gelatinases, and MMP-3 was stromelysin that degrade aggrecan's core protein. A previous study demonstrated that when MMPs were upregulated, PGs, glycosaminoglycans (GAGs), and COL2 were also upregulated (Clutterbuck et al., 2009; Jackson et al., 2006). Upregulated MMPs led to degrade PG and GAGs, therefore, in the osteoarthritis status, the levels of PG and GAGs become to be increased (Jackson et al., 2006). In this study IL-1β induced COL2, PG, and HA. The complex downregulated the expression of COL2, PG, and HA, especially at concentrations 10 and 20 μM of the complex (Fig. 2A, B, and

C). IL-1β noticeably upregulated the production of MMP-1, 2, 3, 9, and 13 and TIMP-1 compared to the control group treated with IL-1β and the complex reduced MMP secretion as well as mRNA expressions at 20 μM (Fig. 2D and Fig. 3A and B). In previous reports, curcumins suppressed MMPs, GAG, and PGE2 at 0.1–50 μM in inflamed chondrocytes (Clutterbuck et al., 2013; Jackson et al., 2006; Schulze-Tanzil et al., 2004). In this study, the complex of curcumin and lecithin enhanced bioavailability of curcumin resulting in chondroprotective effect at relatively lower concentrations. When the complex is administered to human subjects, the dosage could be decreased compared to dosage of independently administered curcumin.

The effects of the complex on IL-1β-induced COX-2 and iNOS expression were also studied. As shown in Fig. 4A, the complex significantly inhibited IL-1β in contrast to the control group. In addition, the anti-inflammatory effects of complex on IL-6, 8 and PGE2 production

Fig. 2 Comparison of COL2 (A), PG (B), HA (C), and TIMP-1 (D) in HCH-c cells stimulated with IL-1 β . Cells were seeded at 1.0×10^5 cells/mL, then stimulated with IL-1 β and sample for 48 h. *

Significant different from the IL-1 β values by ANOVA followed by Dunnett's test ($p < 0.05$)

Fig. 3 Comparison (A) and relative hepatic mRNA expression levels (B) of MMP-1, 2, 3, 9, and 13 in HCH-c cells stimulated with IL-1 β . Cells were seeded at 1.0×10^5 cells/mL, then stimulated with IL-1 β and sample for 48 h. To calculate the relative mRNA expression

levels, the $\Delta\Delta CT$ method was used. The mRNA levels were normalized by β -actin. *Significant different from the IL-1 β values by ANOVA followed by Dunnett's test ($p < 0.05$)

Fig. 4 Relative hepatic mRNA expression levels (A) of the genes COX-2, iNOS and comparison (B) of IL-6, IL-8, PGE2 in HCH-c cells stimulated with IL-1 β . Cells were seeded at 1.0×10^5 cells/mL, then stimulated with IL-1 β and sample for 48 h. To calculate the

relative mRNA expression levels, the $\Delta\Delta CT$ method was used. The mRNA levels were normalized by β -actin. *Significant different from the IL-1 β values by ANOVA followed by Dunnett's test ($p < 0.05$)

were measured. The results demonstrated that IL-1 β noticeably increased the production of IL-6 and -8 and PGE2 in contrast to the control group. The complex decreased IL-6, 8 and PGE2 levels at 20 μ M (Fig. 4B). Mathy-Hartert et al. demonstrated that curcumin (5–20 μ M) decreased the production of cytokines (IL-6 and IL-8), PGE2, NO, and MMP-3 in human chondrocytes when accompanied with IL-1 β (Mathy-Hartert et al., 2009). Similar results showed that curcuminoids (12.5 and 62.5 μ g/mL) decreased mRNA expression of COX-2, iNOS, IL-6, and MMP-3 (Comblain et al., 2015) in human chondrocytes. These results were in agreement with our hypothesis that the complex of curcumin and lecithin maintained potency of curcumin. In conclusion, the complex of curcumin and lecithin showed anti-inflammatory effect similar to curcumin. In addition, the complex might have a chondroprotective effect via an anti-inflammatory mechanism in chondrocytes. As a next step, animal study and well-designed human intervention study to prove enhancing bioavailability as well as efficacy and safety should be performed. Based on this scientific evidence, the complex enhancing bioavailability could be developed as a functional food to improve joint health.

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