Int J Clin Exp Pathol 2016;9(10):10320-10326
www.ijcep.com /ISSN:1936-2625/IJCEP0034372

Original Article

Allicin reduces IL-1β-induced inflammatory cytokines via attenuating the NF-κB and MMP3 activation in human osteoarthritis chondrocytes model

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Received June 24, 2016; Accepted July 9, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Allicin, a sulphur containing compound isolated from the bulb of *Allium sativum*, confirm the various pharmacological activity such as antioxidant and antiinflammatory effects. The current study was intended to evaluate the effect of allicin on the IL-1β-Induced inflammatory cytokines in Human Osteoarthritis Chondrocytes. The chondrocytes were stimulated with IL-1β in the presence or absence of allicin. Moreover, the western blot analyses was used for the estimation of the nuclear factor-κB (NF-κB), c-Jun N-terminal kinase (JNK), cyclooxygenase-2 (COX-2), inhibitory kappa B (IκBα), extracellular signal-regulated kinase (ERK), iNOS and p38 expression. The level of PGE_2 and NO production in the IL-1β treatment were determined via using the ELISA and Griess reagent. In the current study, allicin was found to be significantly inhibited the IL-1β induced iNOS and COX-2 expression in dose dependent manner. On the other hand, allicin in dose-dependent manner causes significant ($P<0.01$) reduction of the IL-1β-induced PGE_2, NO and collagenase-3 (MMP-13) production. Allicin also showed significant reduction of the IL-1β-induced mitogen-activated protein kinase (MAPK) and NF-κB activation. In conclusion, allicin showed efficient attenuation of the osteoarthritis chondrocytes inflammatory response triggered by IL-1β. The result of the current study confirmed that allicin may be beneficial agent in the management or control of osteoarthritis.

Keywords: Allicin, osteoarthritis chondrocytes, COX-2, IL-1β, PGE_2, NF-κB

Introduction

Osteoarthritis (OA) is the common form of arthritis, is a foremost cause of disability amid the elderly [1]. Previous investigation confirmed that incidence of OA increases with the age, for instance; approximately 40% of women and 25% of men was suffered from the OA. The pathologically condition of OA was characterized via development of synovial inflammation, bone remolding and articular cartilage degeneration. Moreover, the clinical symptoms of OA may comprise of increase in stiffness and pain of the joint movement [1, 2]. Previous study showed that, the catabolic and proinflammatory mediators play an imperative role in the expansion of OA incidence, such as, increased level of TNF-α and IL-1β found in the synovial fluid of OA patients. It has been also found that, IL-1β could induce the mitogen activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) activation in the human osteoarthritis chondrocytes. Consequently, the activation of MAPKs and NF-κB initiate the generation of inflammatory mediators viz., COX-2 and NO [3, 4]. The previous investigations confirmed that the inhibition of inflammatory responses induced by IL-1β has the capacity to attenuate the OA expansion [5].

During the OA, the production of interleukin (IL) has been significantly elevated, which causes up-regulation the nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression. It also causes modulation of gene encoding and has profound effect on the secretion of prostaglandin E_2 (PGE_2), nitric acid (NO) in the serum and fluids. Additionally, the arthritic patients also showed increase in the level of NO in the fluid and serum, which is further use as an important indicator of OA pathogenesis [6]. The role of NO has confirmed in the pathogenesis of OA, which is further characterized via inhibition of iNOS in the animal model of OA [7].

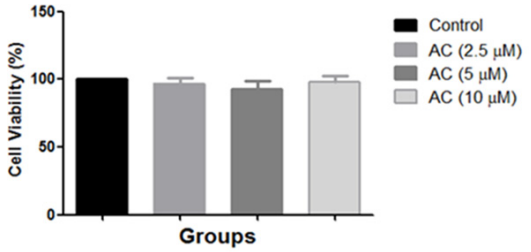

Figure 1. Effects of allicin on the cell viability of chondrocytes. Cells were cultured with different concentrations of allicin (0-10 μM) for 24 h. The cell viability was determined by MTT assay. The values presented are the means ± SEM (n=8) of three independent experiments.

Allicin, a sulfur containing natural product obtained from the *Allium sativum* (garlic), *Allium schoenoprasum*, *Allium cepa* (onion) and 700 other species. Recently, various investigations have confirmed the antioxidant and antibacterial potential of allicin in various different studies. Moreover, several investigations have shown antiinflammatory effect of allicin via inhibition of the various inflammatory cytokines. Whereas, it is surprising to note that, no single study has been attempted to enumerate the effect of allicin in OA disease. Therefore, prompted by the above, in the current study, we make effort to scrutinize the effect of the allicin and enumerate the underlying mechanisms on human osteoarthritis chondrocytes.

Material and methods

Chemicals

Allicin was purchased from the Sigma Aldrich (Sigma Aldrich, USA). All the ELISA kits and recombinant human cytokines were purchased from the approved vendors. All the chemical and reagents were used in the study was of analytical grade.

Tissue collection

The current experimental study was performed according to the Helsinki and Tokyo declaration. In the current experimental study, we have used the human tissue samples. For performing the study, we firstly provide the information to the consents and the investigation was granted form the local ethical committee. The articular cartilage samples were received from the undergoing knee replacement surgery patients (30) and the cartilages were further processed to isolate the primary chondrocytes using the minor modification of previous described method of Ma et al [8].

Cell culture

In the current experimental study, the articular tissues were collected from the non-lesion area and harvested area, which was further processed and cut into small pieces. The minced tissues were treated with the trypsin (25%) for 30 min and again processed with the collagenase II (2 mg/ml) in Dulbecco's Modified Eagle's Medium (DMEM) for 6 h at 37°C. The cells were again mixed in DMEM at 37°C, along with penicillin (100 unit/ml), fetal bovine serum (10%) and CO_2 (5%) [9].

MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was utilized for estimation of effect of allicin on the cellular viability. The various concentration of the allicin was treated with chondrocytes (6×10^3/well) cells seeded in 96 well-plate for 24 h. After 24 h, the medium was removed and each wall were treated with the MTT (5 mg/ml) and additionally cultured for 4 h. After that, each wall was treated with DMSO (150 μl) and the microplate reader was used for the estimation of the absorbance at 570 nm.

Estimation of No

For the estimation of the NO, we have treated allicin with the chondrocytes for 1 h and then stimulated for 6 h with IL-1β. The NO concentration was estimated via the Griess reagent in supernatant in accordance with the manufacturer's instructions.

Western blot analysis and protein extraction

The chondrocytes isolated from the articular tissues was pretreated with the allicin for 1 h and subsequently stimulated with IL-1β for 30 min. The total protein extraction kit was used for extracted the total protein from the chondrocytes and the protein concentration was also estimated via BCA protein assay kit. The proteins (40 μg) were spit on SDS-PAGE (12%) and moved to PVDF membranes.

Statistical analysis

In the current study, the results were showed as means ± SEM. The evaluation between the

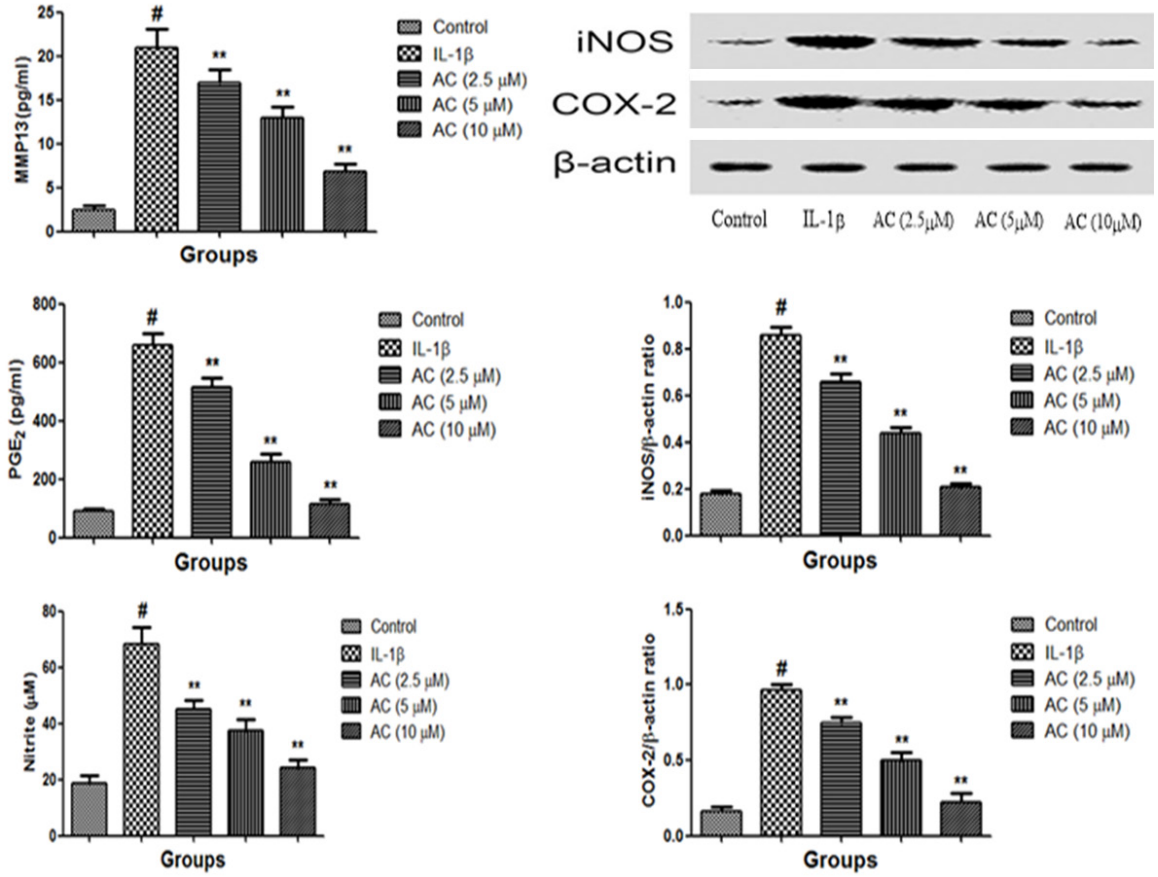

Figure 2. Allicin inhibits MMP-13, PGE_2 and NO production, as well as iNOS and COX-2 expression up-regulated by IL-1β. The data presented are the means ± SEM of three independent experiments, and differences between mean values were assessed by Dennett's test. #P<0.05 vs. control group; **P<0.01 vs. IL-1β group.

groups was done with one way analysis of variance (ANOVA) followed by Dunnett's test, and P<0.05 were considered to indicate statistical significance.

Result

Effect of allicin on cell viability

We firstly scrutinized the effect of the allicin on the cellular viability via MTT assay. As shown in **Figure 1**, no cytotoxicity was observed against the chondrocytes, at the tested dose of 0-10 μM. Therefore, we have selected the dose of allicin (0-10 μM) in the consequent experiments.

Effect of allicin on inflammatory mediators

As shown in **Figure 2**, the effect of the allicin was quantified on the inflammatory mediators including PGE_2, MMP-13 and COX-2. To identify the antiinflammatory effect of allicin, we have evaluated its effect on the PGE_2 production. The **Figure 2** demonstrated significantly (P< 0.05) elevated expression of PGE_2 in IL-1β treatment. Whereas, the IL-1β-induced PGE_2 expression was found to be significantly (P<0.01) inhibited by allicin treatment in a dose dependent way. Several study confirm that the MMP-13 play a crucial roles in cartilage degradation. The current investigation showed that the allicin significantly (P<0.01) reduced the MMP-13 expression induced by IL-1β in dose dependent manner. The **Figure 2** also demonstrated the enhanced expression of COX-2 in IL-1β treatment, which was found to be significantly (P<0.01) suppressed by allicin in concentration dependent manner.

Effect of allicin on IL-1β-induced iNOS and no expression

Several investigations have provided the abundant fact to point out that the iNOS and NO expression plays a momentous function in the

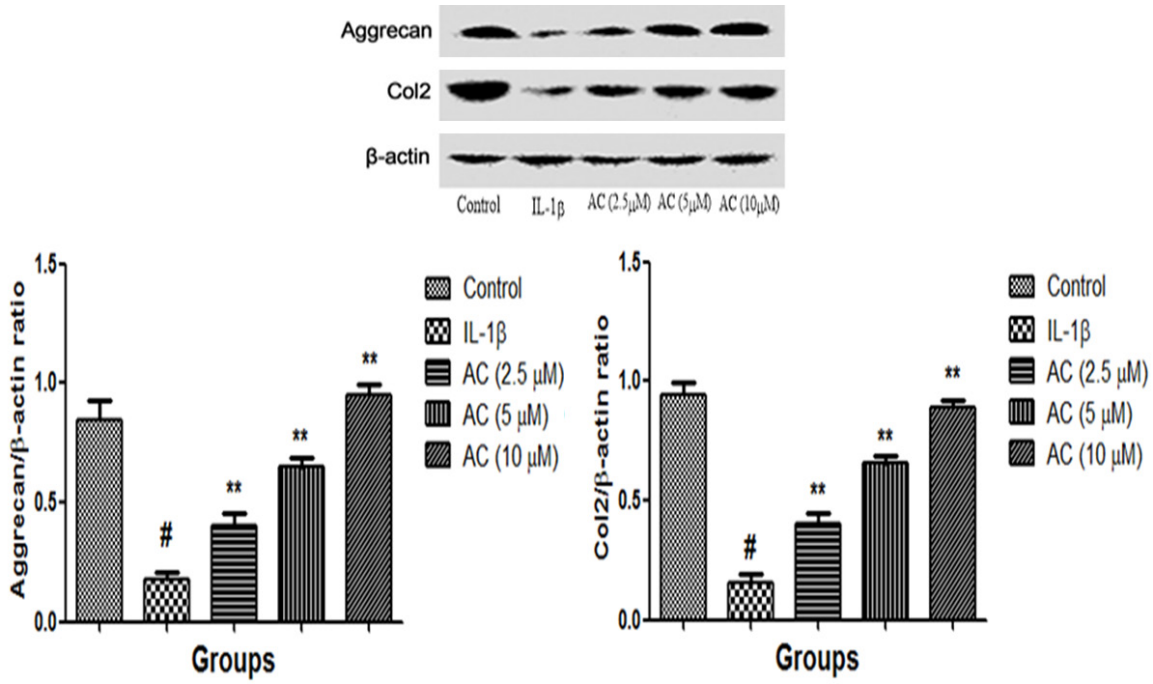

Figure 3. Effects of allicin on aggrecan and type II collagen expression. The data presented are the means ± SEMof three independent experiments, and differences between mean values were assessed by Dennett's test. #P<0.05 vs. control group; **P<0.01 vs. IL-1β group.

expansion of osteoarthritis. Therefore, the effect of allicin on the IL-1β-induced NO was estimated using the Griess reagent. The IL-1β treatment group confirmed the enhanced level of NO in chondrocytes medium and the treatment of the allicin showed the suppressed level of IL-1β-induced NO production in dose dependent manner. The iNOS expression plays a significant role in the development of the inflammation during the NO production. Thus, for the confirmation of the allicin effect on the IL-1β-induced iNOS expression, we have used the western blot technique. In the current study, IL-1β demonstrated the increased iNOS expression, which was found to be significantly reduced by allicin in a concentration dependent manner (**Figure 2**).

Effect of allicin on IL-1β-induced MAPK activation

We first scrutinized the effect of the MAPK, which play a significant role in the generation of inflammatory mediators PGE_2 and NO. In the current study, we have evaluated the effect of allicin on the MAPK activation. The IL-1β-induced showed significant (P<0.05) increase in the MAPK activation, which was found to be significantly (P<0.01) suppressed by allicin (**Figure 3**).

Effect of allicin on NF-κB

In the current investigation, we have scrutinized the activation of NF-κB. In the current study, we have observed the NF-κB activation in IL-1β treatment group (**Figure 4**). Our data revealed the IL-1β-induced activation of NF-κB, which was found to be significantly (P<0.01) reduced the IL-by the allicin treatment in a dose dependent manner.

Effect of allicin on type II collagen and aggrecan expression

In the next instance, we have performed the western blot analysis to scrutinize the type II collagen and aggrecan expression. As presented in **Figure 4**, it has been found that, expression of type II collagen and aggrecan was found to be reduced, which was further significantly (P<0.01) upregualted via allicin treatment in dose dependent manner.

Discussion

OA is a well-known musculoskeletal disease which is characterized via subchondral bone sclerosis and articular cartilage erosion [10]. Several investigations have revealed that, various plant extract and their phytoconstitutents

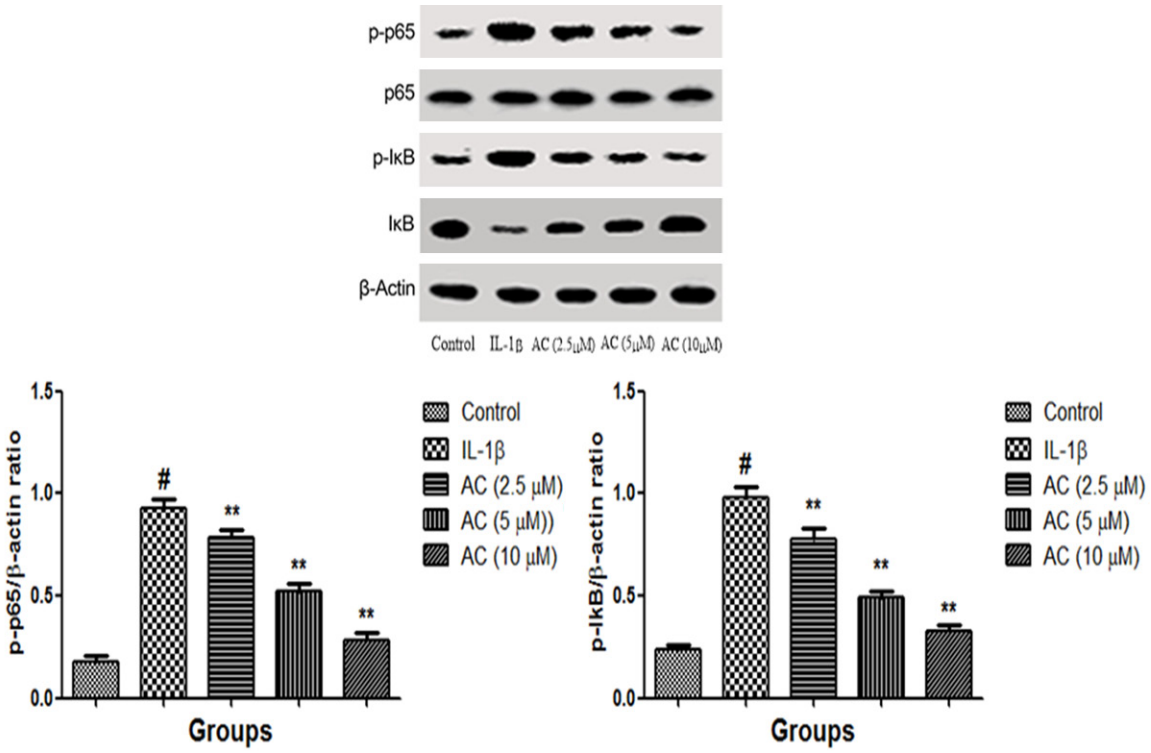

Figure 4. Allicin inhibits IL-1β-induced NF-κB activation and IκBα degradation. The values presented are the means ± SEM of three independent experiments, and differences between mean values were assessed by Dennett's test. #P<0.05 vs. control group; **P<0.01 vs. IL-1β group.

are good entrant for the treatment or control of OA. Among the natural compounds, Allicin (AC), a sulphur containing isolated from the bulb of *Allium sativum*, has been claimed to have anti-inflammatory potential. In the current investigation, we have observed that the allicin significantly causes reduction of the IL-1β induced inflammation. The results of the current study confirm the beneficial effect of allicin against the treatment or control group of the human osteoarthritis chondrocytes.

Various researcher claims that the inflammatory mediators including IL-1β have been reported to play a significant role in the expansion of OA [11]. During the OA, the expression of IL-1β has been increased, which causes elevation of the COX-2 and iNOS expression. It further initiates the production of PGE_2 and NO [12, 17]. The NO has the capacity to generate inflammatory cytokines and induces the production of the PGE_2. Moreover, the enhanced level of the PGE_2 and NO was detected in the patient suffered from OA. The PGE_2 causes degradation of the articular cartilage during the OA, whereas, on the other hand NO promotes the production of PGE_2 and initiate the degeneration of articular cartilage. Earlier studies confirmed, reduction in the level of inflammatory mediators including PGE_2 and NO had the capability to attenuate the OA expansion [13, 14, 18]. In the current experimental study, we have observed that the allicin causes significant (P<0.01) suppression of the production of inflammatory cytokines including PGE_2, NO, as well as COX-2 and iNOS. Few studies have claimed that the MMP-13 deemed to have considerable role in significant part in degradation of cartilage during OA [15]. During experiment IL-1β causes increase in the MMP-13 expression, which was found to be significantly reduced via the allicin treatment in dose dependent manner.

The NF-κB activation directly involved in the regulatory pathway generally initiates the inflammatory processes. NF-κB mostly take part in the regulation of the inflammatory process via activation of the inflammatory cytokines such as PGE_2 and NO. The NF-κB is considered to be responsible for the modulation of the numerous genes during the inflammation and various other mediators which are indicated to be in-

volved in the development of the OA. Under the normal circumstances, NF-κB was bound to the IκB inhibitor and sequester in the cytoplasm [15]. Upon certain stimuli viz., LPS or IL-1β, the NF-κB p65 translocates from the cytoplasm to the nucleus to initiate the production of the various inflammatory mediators viz., iNOS, COX-2, NO and PGE_2. Many other mediators such as MAPKs also play a significant role in the regulation and generation of the inflammatory mediators. The MAPKs expression such as JNK, ERK and P38 are vital mediators in the expansion of inflammation. To confirm the effect of allicin on the inflammation during the OA, we have investigated the effect of the allicin on the LPS induced MAPK and NF-κB activation. The result of the current investigation confirmed that allicin causes significant inhibition of the IL-1β-induced inflammatory response via attenuation of MAPK and NF-κB activation in chondrocytes [16].

In conclusion, the current study confirmed the protective action of allicin via inhibition of the IL-1β-induced production of NO, PGE_2 and MMP-13, as well as causes inhibition of the COX-2 and iNOS expression in chondrocytes via reduction of MAPK and NF-κB activation. The current finding provides the strength to the fact that, allicin might be the beneficial agent in the treatment or control of OA.

Acknowledgements

We acknowledge the Beijing Tiantan Hospital, Capital Medical University for providing the necessary facility to perform this experimental study.

Disclosure of conflict of interest

None.

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