

# EFFECTS OF VITAMIN C ON IL-1 INDUCED NITRIC OXIDE (NO) SYNTHESIS OF ARTICULAR CHONDROCYTES

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## Relevance to Musculoskeletal Conditions

This study examined whether anti-oxidant substances such as vitamin C reduce IL-1-induced nitric oxide synthesis of articular chondrocytes, which might lead to the development of dietary cures against articular cartilage degeneration observed in osteoarthritis and rheumatoid arthritis.

## Introduction

Interleukin-1 (IL-1) plays an important role in cartilage degradation in inflammatory process such as osteoarthritis (OA) and rheumatoid arthritis (RA). It is shown that IL-1 induces the nitric oxide (NO) synthesis of articular chondrocytes in vitro (1). NO implicated in the pathophysiology of inflammatory arthritis, is reported to reduce the formation of extracellular matrix such as proteoglycan and type-II collagen (2,3). Also NO induces the articular chondrocyte apoptosis (4). Thus, these catabolic actions by NO should cause the deterioration of functional property of the articular cartilage.

Recently NF- $\kappa$ B activation by redox reaction is reported to be utilized for the expression of inducible NO synthase (iNOS) mRNA as intracellular signaling pathways from cytokine activation. It may support this phenomenon that an anti-oxidant, pyrrolidine dithiocarbamate (PDTC) suppresses IL-1-induced NO synthesis of articular cartilage due to inactivation of NF- $\kappa$ B (5). Therefore, one of the dietary anti-oxidants, vitamin C is focalized because it plays an important role in modulation of redox reaction both in vitro and in vivo. It is shown that daily subcutaneous administration of vitamin C reduces some symptoms of arthritis animal model (6). High intake of anti-oxidant micronutrients, especially vitamin C, may reduce the risk of cartilage loss and disease progression in people with osteoarthritis (7). In addition to the effect of vitamin C as anti-oxidant, it significantly increased sulfated proteoglycan biosynthesis in chondrocyte cultures in vitro (8). Therefore, in order to elucidate the protective or anabolic role of vitamin C in cartilage metabolism, whether vitamin C may suppress IL-1-induced NO synthesis of articular chondrocyte in vitro was explored.

## Material and Methods

### Cell culture.

Primary chondrocyte monolayer culture was prepared from bovine articular cartilage by collagenase digestion. Cells were suspended in DMEM supplemented with 10% fetal bovine serum and cultured in 24-well plates at  $2.5 \times 10^5$  cells per well for 24 hours. Once the cells have attached to the bottom of culture well, they were treated by 100 U/ml of IL-1 $\alpha$  with or without varying concentrations of ascorbic acid.

### Measurement of NO.

The concentration of NO in the supernatant of the cultured chondrocytes was measured as nitrite concentration by Griess reaction. Briefly, the sample aliquots were mixed with the reaction reagents composed of 0.1 % naphthylethylenediamine dihydrochloride and 1 % sulfanilamide. NO concentration was measured by a spectrophotometer (550 nm) using a standard curve calibrated by sodium nitrite.

### Preparation of RNA (mRNA) and RT-PCR.

For detecting the expression of inducible NO synthase (iNOS) mRNA in articular chondrocytes, RT-PCR was employed using specific primers. Articular chondrocytes were plated at  $1.0 \times 10^6$  cells per well in 6-well plates and stimulated by IL-1 $\alpha$  in the presence of varying concentration of ascorbic acid for 6 h.

Total RNA was directly isolated from the cell monolayers using a RNeasy Mini kit (QUIAGEN, Valencia, CA) according to the manufacturer's instructions. Complementary DNA reverse-transcribed using oligo-dT primer from total RNA was amplified with specific primers using a protocol as reported. A fraction of each PCR products were electrophoresed in a 1.5 % agarose gel followed by ethidium bromide staining and fluorescent intensity was compared with expression of GAPDH mRNA.

## Results and Discussion

When articular chondrocytes were cultured with 100 U/ml of IL-1 $\alpha$  for 48 h incubation, IL-1  $\alpha$  induced a significant increase of nitrite concentration in the articular chondrocyte monolayer culture. Then, addition of 100  $\mu$ M of PDTC abolished this IL-1-induced NO generation. Also, 10  $\mu$ g/ml of ascorbic acid significantly inhibited NO generation of articular chondrocytes in vitro (Fig.1). These observations suggest that ascorbic acid may decrease NO synthesis due to inactivation of NF- $\kappa$ B in articular chondrocytes same as PDTC.

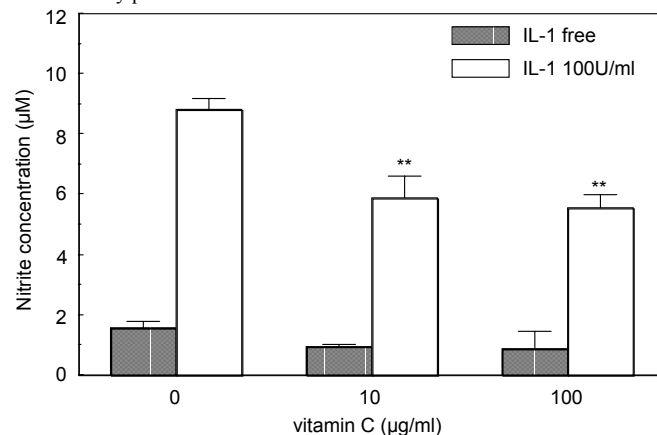
To detect the expression of mRNA, RT-PCR were utilized. The intensity of amplified PCR products from iNOS mRNA was increased by 100 U/ml of IL-1  $\alpha$  treatment. Addition of 100  $\mu$ M of PDTC simultaneously reduced iNOS gene transcription by IL-1  $\alpha$  completely. One mg/ml of ascorbic acid also decreased iNOS gene expression by IL-1  $\alpha$ .

In inflammatory arthritis, there are a lot of proinflammatory cytokines such as IL-1 and they induce NO synthesis of articular chondrocytes. It is known that NO could have a catabolic role in cartilage and promote articular cartilage breakdown. Therefore, regulation of NO synthesis may be one of the promising methods to protect or restore the articular cartilage function.

This is the first report that one of the antioxidant, vitamin C, reduced IL-1-induced NO of articular chondrocytes in vitro. Thus, it is suggested that vitamin C may reduce the risk of cartilage loss and disease progression in people with inflammatory arthritis such as OA and RA.

## Conclusion

Vitamin C may protect the articular cartilage breakdown by NO in inflammatory process.



**Fig.1** Effect of vitamin C on IL-1  $\alpha$  induced NO synthesis from articular chondrocytes. (\*\*:  $p < 0.01$  vs. vitamin C 0  $\mu$ g/ml)

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