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L-Arginine and allopurinol supplementation attenuates inflammatory mediators in human osteoblasts–osteoarthritis cells

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ABSTRACT

This study investigated the synergistic effects of L-arginine and allopurinol on antioxidant and inflammatory mediators in human osteoblasts–osteoarthritis (HOb-OA) cells. The cells were treated with allopurinol (50–150 mg/kg bwt) and L-arginine (50–150 mg/kg bwt) for 72 h. Cell viability, catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), reduced glutathione (GSH), lipid peroxidation, and the inflammatory markers interleukin 6 (IL-6), interleukin 1β (IL-1β), nuclear factor κB (NF-κB) and tumor necrosis factor alpha (TNF-α) were measured. The combined supplementation with allopurinol and L-arginine increased catalase, SOD, GSH, and Gpx, while it decreased lipid peroxidation, IL-6, IL-1β, and TNF-α. While TNF-α, IL-6, IL-1β, and NF-κB mRNA and protein expression were higher in control HOb-OA cells, the combined supplementation with allopurinol and L-arginine substantially reduced their expression in HOb-OA cells by >40%. In summary, combined supplementation with allopurinol and L-arginine might be very effective in osteoarthritis. A search for therapeutic agents that inhibit inflammation could help to prevent and manage osteoarthritis. However, further studies need to determine the biochemical and molecular mechanisms of these agents in osteoarthritis.

1. Introduction

Osteoarthritis is a joint disease that results from the breakdown of bone and joint cartilage [1]. Osteoarthritis is one of the most common types of arthritis and affects 3.3% of the world population [2]. Stiffness and joint pain are the major symptoms of osteoarthritis [3]. The symptoms develop gradually and affect normal daily activities and the ability to work [3]. Inherited genetic factors, abnormal limb development, and joint injury are common causes of osteoarthritis [1]. Berenbaum [4] reported that low-grade inflammation and mechanical stress induce osteoarthritis. Pain medications, regular exercise, and reduction of joint stress are useful treatments for osteoarthritis [5]. Acetaminophen and naproxen are currently used to treat osteoarthritis, but they have many adverse effects, such as gastrointestinal bleeding [6, 7]. There is no definitive treatment for osteoarthritis.

Cells undergo massive oxidative stress due to decreased antioxidants and increased free radical production, and this leads to lipid peroxidation at the cell membranes, cell organelle impairment, and ultrastructural injury [8]. Hsu et al. [9] reported that oxidative stress is involved in the pathogenesis of muscle dysfunction in osteoarthritis, and inhibition of oxidative stress prevents the initiation and development of osteoarthritis. Cifuentes et al. [10] reported the physical training-induced reduction in oxidative stress is effective against osteoarthritis. Several researchers have reported that angiogenesis accompanies chronic synovitis in osteoarthritis and increased synovial vascular density is associated with endothelial cell proliferation [11–13]. Houard et al. [13] reported the role of inflammation and vascular homeostatic mechanism in osteoarthritis.

Allopurinol inhibits xanthine oxidase and is used to reduce the blood uric acid level [14]. Xanthine oxidase is a source of free radicals, which generate oxidative stress and cause chronic tissue damage [15]. Milovanovic et al. [16] reported that L-arginine is an essential amino acid in the biosynthesis of polyamines, urea, creatine, nitric oxide, and proteins and it helps to regulate vascular homeostasis and the cardiovascular systems [17–20]. A preliminary investigation examined the effects of different concentrations of L-arginine on antioxidants and inflammatory mediators in human osteoblasts–osteoarthritis (HOb-OA) cells. However, we did not see a marked effect of L-arginine on HOb-OA cells. Allopurinol is used to slow the progression of OA, since uric acid increases the severity of OA. Therefore, we investigated the

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Table 1
List of primers used in this study.

S. no	Gene name	Sense primer	Anti-sense primer
1	TNF-α	5′-CCCAGACCCTCACACTCAGAT-3′	5′-TTG TCC CTTGAA GAG AAC CTG-3′
2	IL-6	5′-AAGTTTCTCTCCGCAAGATAC TTCCAGCCA-3′	5′-AGG CAAATTTCCTGGTTATATCCA GTTT-3′
3	IL-1β	5′-AGAAGCTTCCACCAATACTC-3	5′-AGCACCTAGTTGTAAGGAAG-3′
4	NF-κB	5′-GAAATTCCTGATCCAGACAAAAAC-3	5′-ATCACTTCAATGGCCTCTGTGTAG-3′
5	GAPDH	5′-TCCCTCAAGATTGTCAGCAA-3′	5′-AGATCCACAACGGATACATT-3′

synergistic effects of L-arginine and allopurinol on antioxidants and inflammatory mediators in HOb-OA cells.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle's medium (DMEM), allopurinol, trypsin-EDTA, L-arginine, antibiotics, and fetal bovine serum (FBS) were obtained from Sigma-Aldrich China (Shanghai, PR China). Fluorescein isothiocyanate (FITC)-conjugated secondary antibody (AP307F, goat anti-rabbit IgG H&L) was from Sigma-Aldrich. The RT-PCR primers were obtained from Thermo Fisher Scientific (Beijing, China).

2.2. Cell culture

HOb-OA cells were purchased from Sigma-Aldrich (406OA-05A SIGMA, Shanghai, PR China). The cells were cultured and grown in standard growth medium (1% antibiotics and 10% FBS) at 37 °C in a CO_2 incubator. The HOb-OA cells were cultured and allowed to reach confluence. Then, the cells were incubated with different concentrations of allopurinol and L-arginine for 72 h. The four experimental treatments were as follows: in Groups I to III, cells treated with L-arginine and allopurinol with doses of 50, 100, or 150 mg/kg bwt of both compounds, respectively; Group IV was a control.

2.3. Marker assays

Cell viability was determined and images were taken under a light microscope [21]. The catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), reduced glutathione (GSH) and lipid peroxidation levels in the cell supernatant were determined [22]. To determine the intracellular ROS level, the medium was removed carefully after the 72-h incubation and the cells were stained with dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 min. The relative fluorescence units (RFUs) were measured under a fluorescent plate reader [23]. At the end of the treatment, the cell supernatant was prepared and the inflammatory cytokines IL-1β, IL-6, and TNF-α were determined [24].

2.4. Real-time polymerase chain reaction

For the quantitative real-time polymerase chain reaction (qRT-PCR) assay, RNA was isolated from the cells and converted into cDNA using oligo(dT) primers. Then, qPCR was used to quantify the mRNA expression with primers specific for IL-1β, TNF-α, IL-6 and NF-κB (Table 1). GAPDH was used as a qPCR internal control. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative ratios of expression [25].

2.5. Western blot analysis

The cell homogenate proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene (PVDF) membranes. Then, the membranes were incubated with primary antibodies against IL-1β, TNF-α, IL-6, and NF-κB for 12 h. Next, the membranes were incubated with horseradish peroxidase (HRP)-IgG (goat anti-rabbit, A0545-1ML, Sigma-Aldrich) for 1 h. The IL-1β, IL-6, TNF-α, and NF-κB protein levels were determined by enhanced chemiluminescence (ECL) [26].

2.6. Immunofluorescence

After 72 h, the cells were incubated with primary antibody against NF-κB for 12 h and then with FITC secondary antibody (ab6840, Abcam) for 1 h [27]. Images were viewed under a fluorescence microscope (BX51, Olympus, Japan).

2.7. Statistical analysis

All of the experimental outcomes are given as the mean with standard error of the mean (SEM). Analysis of variance (ANOVA) was used for multiple comparisons. Statistically, significance was taken when $P < 0.05$.

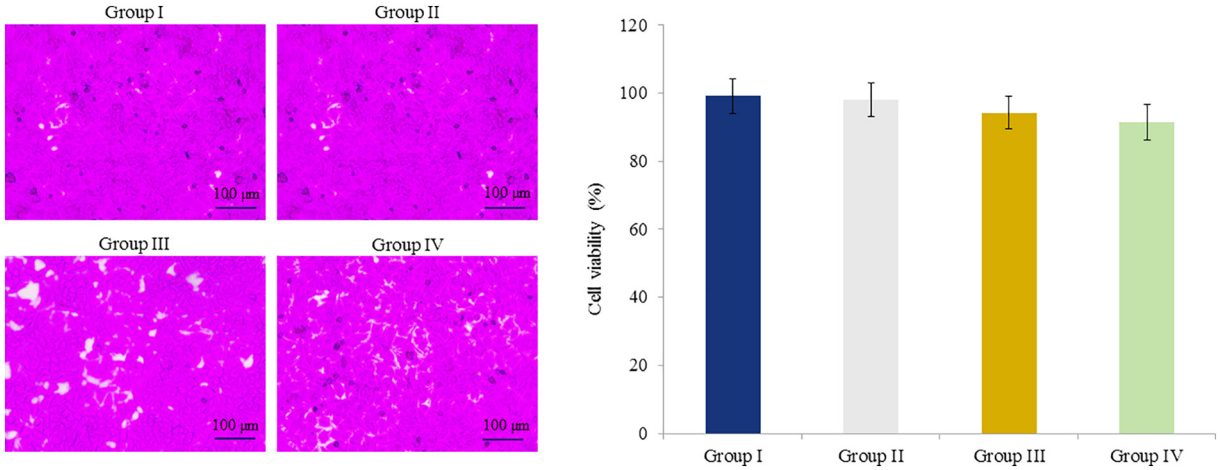

Fig. 1. Synergistic effects of L-arginine and allopurinol on HOb-OA cell viability. The values are expressed as the mean and SEM. The scale bar is 100 μm.