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Potential therapeutic role of Co-Q10 in alleviating intervertebral disc degeneration and suppressing IL-1β-mediated inflammatory reaction in NP cells

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ABSTRACT

Coenzyme Q10 (Co-Q10) is extraordinarily popular and has been used in abundant interventions as an antioxidant reagent that participates in numerous oxidation reactions. According to substantial evidence previously reported, interleukin-1β (IL-1β) is deemed to be one of the chief orchestrator molecules in the degeneration of intervertebral disc (IVD). However, it is unknown whether Co-Q10 is able to protect against IVD degeneration. In the current study, mouse-derived IVDs as well as primary human nucleus pulposus (NP) cells were isolated and cultured. NP cells were stimulated with IL-1β, with or without selective addition of Co-Q10 to investigate the therapeutic effect of Co-Q10 on IVD degeneration. Levels of IL-1β-induced inflammatory biomarkers including TNF-α, COX-2, IL-6 and iNOS were reduced by Co-Q10, which was possibly associated with inhibition of NF-κB signaling activation. Furthermore, Co-Q10 maintained the production of anabolic biomarkers in NP cells such as collagen 2, aggrecan and Sox-9 and altered the enhanced catabolism induced by IL-1β. Moreover, the therapeutic role of Co-Q10 in sustaining IVD tissue-enhanced anabolism is potentially dependent on activation of the Akt signaling pathway. In summary, Co-Q10 may potentially represent an available molecular target that may shed light on approaches to the prevention and treatment of IVD degeneration in the future.

1. Introduction

Intervertebral disc degeneration is considered as an irreversible degenerative disease characterized by alterations in the structure of IVDs, loss of water-binding proteoglycans in NP tissue, degradation of extracellular matrix (ECM) and aggrecan, and diminished synthesis of type 2 collagen (Col 2) [1]. These modifications subsequently accelerate a reduction in disc height and induce the development of disordered homeostasis of IVD tissue metabolism [2]. Currently, the primary measure for IVD degeneration is often surgical intervention. Therefore, the discovery of additional mechanisms associated with IVD degeneration and target treatments is urgently needed.

It is known that unbalanced anabolism in NP cells is a characteristic change during IVD degeneration [3]. As previously reported, decreased expression of aggrecan and Col 2, two critical matrix components of IVD tissue suggests the progression of degeneration in IVD [4]. Additionally, mechanisms that contribute to the enhancement of anabolism in NP tissue provide a possible therapeutic target for intervention in IVD disease. It is interesting to speculate how maintaining the production of ECM in NP tissue can have a pivotal effect on the prevention of IVD degeneration [5]. An earlier study described increased catabolic activity in IVD degeneration, which is closely correlated with IL-1β-linked enhanced expression of various metalloproteinases [6], namely, a disintegrin and metalloproteinase containing both thrombospond in motifs 5 (ADAMTS-5) and MMP-13 [7–9].

It is known that IL-1β activates the NF-κB signaling pathway and induces the production of numerous inflammatory molecules, resulting in typical cell apoptosis, proliferation, and synthesis of a fibrotic matrix and consequently leading to more severe developmental inflammatory responses surrounding the IVD tissue. This process suggests that a feasible approach to alleviating IVD degeneration involves the use of factors that interfere with the IL-1β signaling pathway [6].

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Coenzyme Q10 (Co-Q10) is a multifunctional molecule that reportedly plays a protective role in numerous physiological and pathological conditions including gastric ulcers, regeneration, and chondrogenesis [10–12]. In recent years, several studies have demonstrated that Co-Q10 serves an anti-inflammatory function, and on a molecular level, effectively suppresses the production of both IL-1β and TNF-α [13,14]. On a macroscopic level, Co-Q10 has been found to attenuate dermatitis, osteoarthritis (OA) and inflammatory bowel diseases [15]. Since inflammatory molecules such as IL-1β mediate various inflammatory responses, scholars have investigated whether rCo-Q10 antagonizes IL-1β and thereby plays a therapeutic role in decreasing the morbidity associated with OA [14]. Progressive increases in the quantity of IL-1β induce an exaggerated activation of the NF-κB signaling pathway, which significantly exacerbates IVD degeneration and increases insufferable ailments [16]. Moreover, related studies have revealed that the Akt signaling pathway is considered a pivotal channel in the anabolism of NP cells [17,18]. As demonstrated by several previous studies, Co-Q10 suppresses the activation of the NF-κB signaling pathway and promotes the activation of the Akt signaling pathway in various diseases [19,20]. Considering all of the above, we designed this study to examine the potential interactions of Co-Q10 in the treatment of IL-1β-stimulated IVD tissues and primary human NP cells. To further investigate results originating from earlier studies, we sought to determine whether the NF-κB signaling pathway, as well as the Akt signaling pathway, is regulated by Co-Q10 and involved in IVD degenerative disease.

2. Materials and methods

2.1. Ethics statement

In this study, all human IVD fragments were obtained from 48 patients who underwent lumbar surgery (November 2013to May 2017) in Qilu Hospital of Shandong University, Jinan, China. Patients involved in this study were all given informed consent documents and voluntarily agreed to participate in this research. All animal experimental procedures were performed in accordance with the International Guiding Principles for Animal Research and were approved by the Laboratory Animal Centre of Shandong University. Wild-type (WT) mice (3 months old, $n = 8$) were used in this study.

2.2. Ex vivo murine IVD isolation and culture

In this study, all surgical procedures were performed under sterile conditions. The lumbar spines were removed from sacrificed mice, and IVD tissues were dissected within 12 h after sacrifice for ex vivo culture [21]. As briefly described, all murine lumbar discs after separation were incubated in DMEM/Ham's F-12 medium (Hyclone, Thermo Co., USA) plus 1% mini-ITS, and then, after culturing for 24 h, felicitous discs were incubated and stimulated withIL-1β (10 ng/mL) for 7 days, simultaneously, with or without treatment with 20 μM Co-Q10. After each loading period, all IVD tissues cultured ex vivo were collected, and the following experiments were performed.

2.3. Primary human NP cell isolation and culture

Following surgical discectomy, human IVD samples were retrieved from 48 patients (aged 23–54) with lumbar disc degenerative disease. In this study, all the procedures involving the isolation and culture of primary NP cells were performed as previously reported [22]. Human disc tissues were transferred to the laboratory immediately after surgery was completed. Residual blood was removed by fully washing the discs with cold, aseptic phosphate-buffered saline (PBS). Then, the end plate cartilage/annulus fibrosus was carefully removed, and NP tissues were separated and cut into fragments of approximately 1 mm^3. The tissue samples were separately digested with trypsin for 1 h followed by digestion with type II collagenase for 4 h (Sigma-Aldrich, Ltd., China). Primary NP cells obtained by using a septic technique were filtered through a 200-mesh sieve, and the remaining tissue fragments were removed by filtration through a 100 μm cell strainer. The isolated NP cells were seeded as a monolayer and cultured in DMEM/F12 medium (HyClone, Thermo Co., USA) containing 15% FCS and 1% PS under standard incubation conditions (37 °C, 95% air, 5% CO_2, bicarbonate buffer to maintain pH 7.2) for approximately 3 weeks. The culture media was replaced once every 3 days. All cells were passaged when they reached approximately 80–90% confluence, and the indicated experiments were then carried out.

2.4. Histology and immunohistochemistry

For each ex vivo experimental group, after stimulation with IL-1β, IVD tissues were fixed in 4% PFA for 3 days. To decalcify the IVD tissues, the samples were immersed in 10% *w/v* EDTA for 2 weeks before being dehydrated and embedded in paraffin. After all of the above steps were completed, the tissues were cut into 5 μm thick sections. Partial sections were stained with Safranin-O/fast green/iron hematoxylin stain. Moreover, additional human IVD tissues were incubated in Tris buffer (10 Mm Tris-HCl (pH 8.0), 150 mM NaCl) and prepared for immunohistochemistry. The expression of MMP-13 and IκB-α in IVD sections was evaluated by using anti-MMP13 antibody (1:200 dilution, ab3208, Abcam) and anti-phosphorylated IκB-α antibody, (pIκB-α, sc-7977, diluted 1:1000), respectively, and then incubated at 4 °C overnight following the manufacturer's recommendations. Then, the sections were incubated with biotinylated anti-rabbit IgG (Vector, Burlingame, CA) for 30 min (37 °C). Subsequent staining was performed using a biotin-streptavidin-peroxidase protocol (Vector). Horseradishperoxidase (HRP) activity was detected using 3,3′-diaminobenzidine and H_2O_2, and the slides were counterstained with 0.5% methyl green. The sections were examined under a light microscope.

2.5. Protein extraction and western blot

Protein was collected from each indicated experimental group, as previously reported [23]. Briefly, equivalent quantities of protein from each group were calibrated and incubated with primary polyclonal anti-iNOS (sc-7271, diluted 1:1000), anti-collagen 2 (sc-52,658, diluted 1:1000), anti-phosphorylated IκB-α (pIκB-α, sc-7977, diluted 1:1000), COX-2(diluted 1:1000, Santa Cruz Biotechnology) or anti- NF-κB p65 (ab97726) for 1 h (37 °C). After washing with TBST 3 times, the samples were incubated with an alkaline phosphatase–coupled secondary antibody (horseradish peroxidase-conjugated anti-rabbit immunoglobulin; 1:2000 dilution), and using an enhanced chemiluminescence system (Amersham Life Science, Arlington Heights, IL, USA), bound antibody was visualized. The expression of cytoplasmic protein was normalized to β-Actin using ImageJ software.

2.6. Real-time PCR

Total mRNA was isolated from both primary NP cells and cultured murine IVD tissue from each indicated group using an RNeasy kit (Qiagen). Reactions were performed with SYBR Green I dye used to monitor DNA synthesis. GAPDH was used as an internal control to normalize the expression of numerous genes. For amplification, primers used for real-time PCR were designed. Sequence-specific primers used in the present study are listed in Supplementary Tables 1 and 2.

2.7. Reporter gene assay

Since Co-Q10 inhibition of IL-1β-mediated transactivation of NF-κB-dependent reporter genes was considered in this study, a reporter gene assay was performed, as previously described [24]. Briefly, NP cells grown to ~50% confluence were transfected with 1 μg of the p6XNF-