Bisphosphonate Rescues Cartilage from Trauma Damage by Promoting Mechanical Sensitivity and Calcium Signaling in Chondrocytes

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Introduction: Our previous study found that systemic injection of zoledronic acid (ZA), a bisphosphonate for bone loss treatment, could suppress the development of post-traumatic osteoarthritis (PTOA) in the DMM (destabilization of medial meniscus) mouse model (Fig 1) [1]. However, little is known about the chondroprotective mechanism of this FDA approved drug. In this study, we hypothesized that 1) the presence of ZA could rescue the cartilage from traumatic damage under in vitro culture, 2) the supplement of ZA in culture medium could promote the beneficial effects of mechanical stimulation on cartilage explant culture, and 3) the chondroprotective effects of ZA is related to the spontaneous calcium signaling in chondrocytes.

Methods: Rescue from Serum Damage: Cartilage allografts from 3-month old calf knee joints were cultured in serum supplemented medium (DMEM, 10% FBS, 1% P/S) for 1 week to simulate traumatic damage on cartilage caused by joint bleeding. In our previous study, mechanical integrity of cartilage allografts was significantly impaired after exposure to serum [2]. The samples were further divided into two groups and cultured in chemically defined serum-free medium [3] for 4 weeks with or without 1 μM ZA. Serum-free medium includes DMEM, 1% ITS, 50 μg/mL L-proline, 0.9 mM sodium pyruvate, dexamethasone (0.1 μM), and ascorbate 2-phosphate (50 μg/mL). Longitudinal mechanical properties of the allografts were measured weekly, followed by GAG and collagen assay at the end of the 4-week culture. Cartilage allografts were cut into halves, stained with Fluo-8 AM and imaged on a confocal microscope (Zeiss LSM510) for 15 minutes. Spontaneous calcium responses of in situ chondrocytes were recorded at day 1, 8, 15, and 29.

Mechanical Stimulation: After harvesting, cartilage allografts were cultured in serum-free medium with or without the presence of 1 μM ZA for 4 weeks. Samples were stimulated with mechanical loading (10% preload followed by ±3% dynamic loading) for 30 minutes every day. Mechanical properties, equilibrium young’s modulus and dynamic modulus, of allografts were measured weekly. Gene expressions of aggrecan (AGN), type I collagen (COL1) and type II collagen (COL2) were tested using qRT-PCR after 2 weeks of culture. Biochemical assays were done to measure the GAG and collagen contents after 4 weeks of culture.

Results: For cartilage allografts initially exposed to serum for 1 week, the Young’s modulus of the ZA group increased more than in vitro exposure induced damage in cartilage allografts, which implies that ZA may rescue the PTOA by acting directly on chondrocytes. This conclusion is further strengthened by the fact that ZA treatment can promote the benefit of mechanical stimulation in cartilage explant culture.

Discussion: Systemic injection of bisphosphonate can rescue PTOA in animal model, which suggests a promising therapeutic technique for trauma related cartilage degeneration. Our in vitro experiments showed that treatment with ZA can rescue the serum-exposure induced damage in cartilage allografts, which implies that ZA may rescue the PTOA by acting directly on chondrocytes. This conclusion is further strengthened by the fact that ZA treatment can promote the benefit of mechanical stimulation in cartilage explant culture.

Significance: We have shown that ZA, an FDA approved drug for the treatment of bone disease, can rescue the cartilage from trauma damage by acting directly on chondrocytes. This chondro-protective effect of ZA is correlated with the intracellular calcium signaling in chondrocytes.

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Fig. 1: Continuous administration of the FDA approved drug zolendronic acid (ZA), a bisphosphonate to treat bone loss, suppressed the development of post-traumatic OA in a DMM mouse model (destabilization of the medial meniscus). A) Histological analysis and B) Cartilage Damage Scoring of DMM and ZA treated mouse knees. (Veh: Vehicle control group)

Fig. 2: A) GAG content and (B) collagen content of cartilage allografts; (C) Responsive percentage of spontaneous calcium signaling in chondrocytes at day 2, 8, and 15 after serum exposure; (D) Normalized equilibrium Young’s modulus and (D) dynamic modulus of the ZA rescued and non-ZA groups (n = 12).
Fig. 3: Live/Dead staining of samples under mechanical stimulation on day 28 (Scale bars=50μm).

Fig. 4: (A) GAG content and (B) collagen content of cartilage allografts with 4-week mechanical stimulation; (C) Young’s modulus and (D) dynamic modulus of the ZA treated and non-ZA groups (n = 12).
Fig. 5: Chondrogenesis gene expression of chondrocytes in cartilage allografts with mechanical stimulation: (A) Type I Collagen, (B) Type II Collagen, and (C) Aggrecan.