Intermittent hypoxic stimulation exerts chondroprotective effects on cultured chondrocytes and in a rat osteoarthritis model

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INTRODUCTION: Hypoxia-inducible factor (HIF)-1α is activated under hypoxic conditions and exerts chondroprotective effects in articular cartilage. Its expression is influenced by the duration and pattern of hypoxic stimulation. Previous studies using tumor and vascular endothelial cells have shown that intermittent hypoxic stimulation induces HIF-1α more effectively than continuous hypoxic stimulation (Figure 1). We hypothesized that intermittent hypoxic stimulation could enhance HIF-1α expression in chondrocytes and may offer therapeutic benefits for osteoarthritis (OA). This study aimed to investigate the effects of intermittent hypoxic stimulation on chondrocytes and in a rat OA model.

METHODS: Human chondrocytes were cultured under alternating hypoxic and normoxic conditions for a total of 8 hours. As a control, chondrocytes were cultured under continuous hypoxia for the same duration. Protein expression of HIF-1α, SOX9, and aggrecan was evaluated by Western blotting after exposure to the respective oxygen environments. Gene expression of HIF-1α, SOX9, ACAN, ADAMTS4, and MMP13 was evaluated by RT-PCR at three time points: before stimulation, after continuous hypoxic stimulation, and after intermittent hypoxic stimulation. In the animal model, rats with OA induced by intra-articular injection of 0.5 mg monosodium iodoacetate (MIA) were divided into two groups (n=10 each): one exposed to a normoxic environment and the other to an intermittent hypoxic environment (alternating every 24 hours). All rats were male to eliminate the influence of hormonal factors. Histological evaluation was performed after 4 weeks. All animal procedures were approved by the Experimental Animal Committee of Kyoto Prefectural University of Medicine (No. M2020-533).

RESULTS: Protein expression levels of HIF-1α, SOX9, and aggrecan in chondrocytes significantly increased with each cycle of intermittent hypoxia, and their final expression levels were higher than those observed with continuous hypoxia (Figure 2). Gene expression levels of SOX9 in chondrocytes of intermittent hypoxia stimulation group were significantly increased compared to other groups. HIF-1α, SOX9, ACAN, ADAMTS4, and MMP13 gene expression levels were not significantly different. In the OA rat model, articular cartilage degeneration was significantly reduced in the intermittent hypoxia group compared to the normoxia group (Figure 3).

DISCUSSION: It is known that HIF-1α expression increases under acute hypoxia but is downregulated during prolonged hypoxia due to a negative feedback mechanism involving prolyl hydroxylase domain enzymes (PHDs). In our study, intermittent hypoxic stimulation repeatedly enhanced HIF-1α expression and upregulated downstream factors SOX9 and aggrecan, indicating increased cartilage metabolic activity. Histologically, cartilage degradation in the OA model was significantly suppressed by intermittent hypoxia. These findings suggest that intermittent hypoxic stimulation effectively induces HIF-1α and may offer a novel chondroprotective strategy for OA.

SIGNIFICANCE/CLINICAL RELEVANCE: Intermittent hypoxic stimulation enhances HIF-1α expression in chondrocytes and suppresses cartilage degeneration in OA. This strategy could represent a novel, non-invasive therapeutic approach for OA.

IMAGES AND TABLES:

Figure1

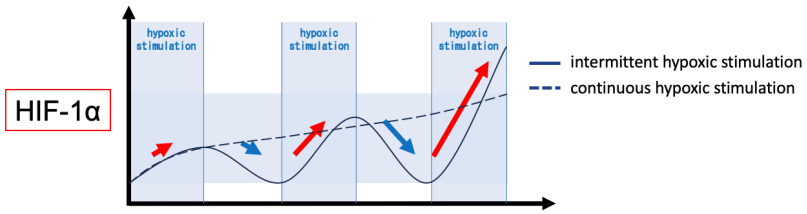

Figure2

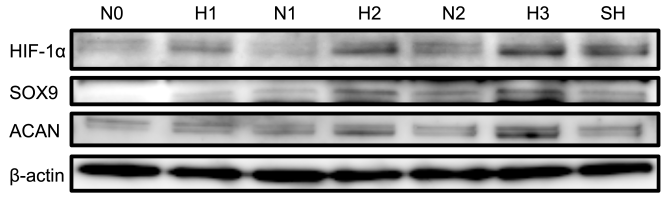

Figure3