GLUCOSAMINE PROMOTES CHONDROGENIC PHENOTYPE IN BOTH CHONDROCYTES AND MESENCHYMAL STEM CELLS AND INHIBITS IL-1BETA INDUCED MMP-13 EXPRESSION AND MATRIX DEGRADATION

*Derfoul, A; *Miyoshi, A, +*Tuan, RS., +*Cartilage Biology and Orthopaedics Branch, NIAMS-NIH, Bethesda, MD 20892

**Introduction:** A common feature of the arthritic cartilage is the to be of matrix components including collagen and proteoglycans, such as aggrecan, due to accelerated turnover and inadequate repair. Glucosamine (GlcN) is a constituent of articular glycosaminoglycan (GAG), and GlcN salts constitute a new class of drugs that have been shown in several clinical trials to modify disease symptoms and promote cartilage repair (1). Adult human articular cartilage has been shown recently to contain mesenchymal stem cells capable of chondrogenic differentiation (2). We hypothesize that GlcN may act by blocking cartilage degradation and by altering adult mesenchymal progenitor cell function during normal cartilage turn over and repair. To test this hypothesis we examined the effects of GlcN chloride on human osteoarthritic (OA) articular chondrocytes, activated with interleukin 1-beta (IL-1beta). In addition, we investigated GlcN effect on the differentiation characteristics of a human multi-potential mesenchymal progenitor cell line (hMSCs). These cells can differentiate into chondrogenic phenotypes, in defined medium supplemented with dexamethasone plus TGF-beta, when cultured at high density (3, 4). Here, we report that GlcN treatment enhanced chondrogenic differentiation of hMSCs and OA chondrocytes, as shown by upregulation of the expression of cartilage matrix components aggrecan and collagen II (Col II), blocked IL-1beta induced matrix metalloproteinase 13 (MMP13) expression in both normal and OA chondrocytes, and enhanced aggrecan and sulfated GAG levels in OA chondrocytes. These results strongly suggest that glucosamine has chondroinductive and chondroprotective effects on normal and OA chondrocytes as well as hMSCs.

**Methods:** Primary OA chondrocytes were isolated from knee articular cartilage obtained from George Washington University from patients undergoing knee arthroplasty. Normal human articular chondrocytes were purchased (Cambrex). A multi-potential mesenchymal progenitor cell line (hMSCs) was derived from trabecular bone (3). Primary chondrocytes were grown as high density pellet cultures by pelleting 250 000 cells in 15 ml conical tube s in chondrogenic medium consisting of high glucose DMEM serum free - medium supplemented with ITS, 100 µg/ml sodium pyruvate, 40 µg/ml L- proline and 50 µg/ml of L-ascorbic acid 2-phosphate. Cells were treated for 24 h with 5ng/ml IL-1beta, then washed and treated with 1 mM GlcN for an additional 10 days. hMSCs were grown as high density pellet cultures in chondrogenic medium supplemented with 100 nM DEX and 10 ng/ml of recombinant human TGF-beta 3 and 3 treated at day 4 in the presence or absence of 100 µM GlcN. Cultures were carried out for 11 or 21 days. Cells harvested from pellet cultures were processed for RNA extraction followed by RT-PCR, immunohistochemical analyses, alcian blue staining or GAG assays performed according to standard procedures. Specific primers for aggrecan, collagen II (Col II), and glyceraldehydes 3-phosphate dehydrogenase (G3PDH) were designed based on the corresponding GenBank cDNA sequence. Specific antibodies to human aggrecan and Col II were purchased (Developmental Hybridoma).

**Results:** 100 µM GlcN treatment of high-density hMSC aggregates, from day 4 to day 21, significantly enhanced the expression of chondrogenic marker genes, aggrecan and Col II, compared to control untreated pellets. Similar upregulation of these genes was observed in OA chondrocytes (Figure 1). Collogen type II protein immunostaining and alcian blue staining was observed in sections of pellet cultures treated with 100 µM GlcN. However, higher doses of GlcN (10 mM and 20 mM) were inhibitory to both chondrocytes and hMSC chondrogenesis (data not shown). Treatment of OA chondrocytes with IL-1 beta enhanced basal levels of MMP13 and induced a strong expression of MMP13 in normal chondrocytes, which otherwise do not express any MMP13 (figure 2). This IL-1 beta effect was completely blocked in normal chondrocytes and partially reversed in OA chondrocytes, following GlcN treatment. Real-time RT-PCR analysis showed that GlcN inhibited MMP-13 and enhanced aggrecan expression by 2 fold, in IL-1beta treated OA chondrocytes (Figure 3). In addition, GlcN treatment reversed IL-1beta-induced reduction of sulfated GAG levels in OA chondrocytes (Figure 4).

**Discussion:** Current treatment of arthritis is limited to nonsteroidal anti-inflammatory drugs, injectable intraarticular corticosteroids and hyaluronic acid, which provide pain relief but do not interfere with the progression of the disease. This data suggest that GlcN enhances chondrogenic differentiation of hMSCs by upregulating expression of aggrecan and Col II. In addition GlcN promotes the chondrocytic phenotype by enhancing sulfated proteoglycan accumulation and blocks cartilage matrix degradation by inhibiting MMP13 expression. Enhancing hMSC chondrogenesis in combination with blocking cartilage degradation may account for the putative chondroprotective properties of GlcN. Understanding the mechanism of action of GlcN on mesenchymal differentiation and cartilage repair should lead to better management and prevention of inflammatory diseases affecting skeletal function and repair.

**References**


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