Glucosamine modulated changes in articular cartilage detected by $T_{1\rho}$ Imaging

S. V. Akella[1], M. Corbo[1], G. R. Dodge[2], A. J. Wheaton[1], R. R. Regatte[1], R. Reddy[1]

[1]Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Bone and Cartilage Research Laboratory, Alfred I. duPont Hospital for Children, Wilmington, DE, United States

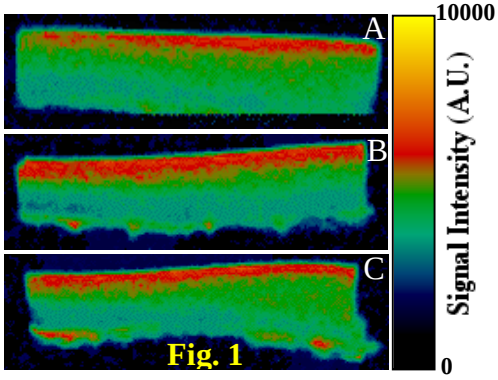
Introduction

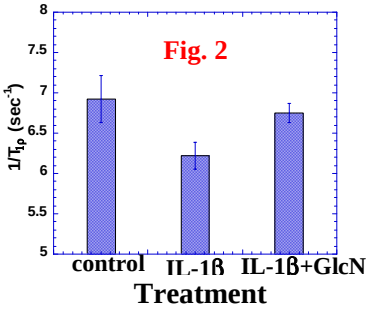
Osteoarthritis (OA) is characterized by the progressive degradation of articular cartilage and an eventual loss of function leading to arthroplasty. Recently glusosamine (GlcN) has become a popular nutritional supplement for the treatment of OA in humans (1). GlcN is a naturally occurring, non toxic compound that, when given orally has been shown to decrease pain and improve mobility in osteoarthritic joints of humans. Numerous recent studies show that GlcN can modify cartilage PG biosynthesis by increasing the expression of PG, such as aggrecan and on IL-1 induced pathways of PG degradation.. Recently our group has shown that GlcN was preferentially incorporated into the galactosamine moiety of chondroitin sulfates in articular cartilage explants (2). It is already reported that $T_{1\rho}$ relaxation is a sensitive marker for changes in proteoglycan (PG) content (3). We hypothesize that modifications in the PG content with the treatment of Interleukin–1β (IL–1β) and GlcN can be monitored by $T_{1\rho}$ relaxation. In the present study we investigated the effect of GlcN on $T_{1\rho}$ relaxation of articular cartilage treated with IL–1β and monitored any changes in PG content by $T_{1\rho}$ relaxation and dimethylmethylene blue assay (DMMB) .

Methods

Femoral joints from 1-2 year old steers were obtained from local slaughterhouse within few hours of animal sacrifice with IACUC-approved exemption. One-cm diameter cartilage plugs were cored from them and equilibrated in phosphate buffered saline (PBS). The samples were divided into three groups of 5 specimens each. 6 ml of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (DMEM) with or without IL-1β alone or IL-1β plus glucosamine was used. The first group of samples were treated with control medium continuously throughout the 10 days, the second group with IL-1β for the first 4 days and then plain DMEM for the remaining 6 days, and the third group received IL-1β for the first 4 days and 2 mg/ml GlcN medium from the 4th day to the 10th day. The samples were incubated at 37°C under 5%CO_2/95% air. At the end of the culture, the explants were equilibrated in PBS until MR measurements. $T_{1\rho}$ imaging experiments were performed on 4.7 T magnet interfaced to a Varian INOVA console using a linearly driven radiofrequency birdcage coil. The pulse sequence contains a three pulse cluster consisting of two 90° hard pulses separated by a 90° phase shifted, low power, long rectangular pulse for spin locking pre-encoded to a fast spin echo (FSE) sequence (4). Imaging data was processed with the IDL language. $T_{1\rho}$ relaxation was calculated by fitting the signal intensity to an exponential equation. The treated explants were subjected to papain digestion and measured the PG content in the tissue by DMMB assay

Results & Discussion





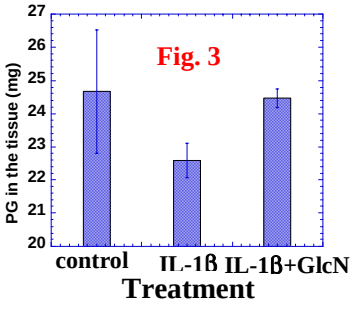


Figure 1 shows the $T_{1\rho}$ images of control (Fig.1A), IL-1β treated (Fig. 1B) and IL1β+GlcN treated (Fig. 1C) cartilage explants at a TSL of 80 ms and spin lock frequency of 500 Hz. In the images, the yellow and red colors indicate regions of low PG, high water contents and higher $T_{1\rho}$ numbers. The average $T_{1\rho}$ relaxation value in control cartilage explants is 145±6.1 ms, whereas in IL-1β degraded explants is 161±4.4 ms and in IL-1β+GlcN treated explants is 148±2.6 ms, which indicates that the amount of PG content lost as a result of IL-1β degradation is replenished with the treatment of 2 mg/ml GlcN. In **figure 2**, the average $T_{1\rho}$ relaxation rate is plotted for three groups of samples with error bars indicating the standard deviation in $T_{1\rho}$. Low $T_{1\rho}$ relaxation rate of IL-1β treated explants indicates the amount of PG loss. In the **figure 3**, amount of mean PG in the tissue measured by biochemical assay is plotted for the three groups of explants. The increase in PG content in the IL-1β+GlcN treated samples in comparison to IL-1β treated samples is due to the effect of GlcN.

Conclusion

These initial experiment show that $T_{1\rho}$ relaxation can detect small changes in cartilage PG and that the PG lost by IL-1β treatment can be restored if the cultures are treated with GlcN. This report demonstrates that changes in PG can be monitored by $T_{1\rho}$ relaxation and that this technique has potential to be utilized to monitor the effectiveness of therapies in joint diseases where early diagnosis is crucial. Work is in progress to measure the effect of GlcN in a dose-dependent manner and correlate the changes in PG in articular cartilage with $T_{1\rho}$ relaxation.

Acknowledgements

This research was supported by NIH grants RR02305, AR45242 and arthritis foundation.

References

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