A COMPARATIVE STUDY OF DEPROTEINIZED HUMAN SYNOVIAL FLUID IN EARLY AND LATE STAGE OSTEOARTHRITIS USING 500MHZ 1H-NMR SPECTROSCOPY

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Introduction

Severe, irreversible tissue damage occurs early in the osteoarthritic (OA) disease process, prior to the onset of radiographic changes. The degradation products, enzymes, and signal transduction molecules involved in OA are first released from the cartilage matrix into the synovial fluid. Joint fluid analysis should, therefore, be able to provide biochemical information about the metabolic state of a specific joint. As one of the most powerful methods in analytical chemistry, high resolution nuclear magnetic resonance spectroscopy (NMR/MRS) is particularly well suited to the assessment and quantification of a wide variety of low molecular weight metabolites present in body fluids such as synovial fluid (SF). In principle, quantitative ¹H MRS data can be recorded from all proton containing metabolites of interest in one single-pulse experiment. However, in practice, the intense and irregular background resonances from the macromolecular of SF (albumin, proteins, and long-chain polymers) obscure the metabolites of interest and render quantification unreliable; therefore, special quantification approaches must be adopted. Deproteinization using centrifugal ultra-filtration represents a particularly efficient means by which to remove protein interference, thus presenting a powerful method by which to measure the concentrations of low molecular weight metabolite species. Ultrafiltration also serves to free the SF samples of macromolecules and cellular debris that are hidden by more dominant resonances. The significant increase in β-hydroxybutyrate and acetate in severe with respect to mild OA SF is consistent with the hypothesis suggesting increased fatty acid utilization with OA state, since both substances are ketone byproducts of lipolysis. Alternately, these metabolites may be derived from the presence of an immunological infiltration; recent work has shown that lymphocyte metabolism is largely attributable to the oxidation of ketones.

Methods

Five 1 ml SF samples from each of the above categories (previously obtained from patients undergoing arthroscopic debridement for treatment of OA and stored at -75°C) were made in a 60:40 ratio of filtrate: D₂O in 5 mm NMR tubes for a total control volume of 600 µl. Samples were filtered through 30 kD molecular-weight-cut-off (MWCO) centrifugal filters (Amicon, USA) for 12-15 hours at 3000 g. The filters were all repeatedly (12 times) rinsed with 2 ml distilled H₂O (8 times) to remove any traces of the glycerine coating covering the filter membranes. Data were acquired at 500.011 MHz ¹H using 90° pulses, 256 averages, a 5 sec delay, 3 sec acquisition time, 16, 384 points, 6000 Hz spectral width and using gated presaturation at the water resonance frequency to suppress the intense water signal. Chemical shifts were referenced to a known concentration external-standard of TSP contained inside a co-axial capillary tube at the center of each NMR sample. Peak areas obtained using a Marquardt-Levenberg line fitting routine/integration were normalized with respect to the peak area from the TSP. Changes in metabolic profiles of the SF samples were assessed for the following metabolites: glucose, choline, creatinine, creatine, citrate, pyruvate, acetate, alanine, lactate, β-hydroxybutyrate, valine and leucine. Statistical significance was tested using Student’s t-tests.

Results

Several metabolites were found to show significant changes with disease severity. In particular, citrate, 3-D-hydroxybutyrate, lactate, formate, sugars/polyols, and threonine demonstrated strong differences (p<0.05).

Discussion

The combination of ultrafiltration and high resolution ¹H NMR analysis is a particularly powerful method by which to measure the concentrations of low molecular weight metabolite species. Ultrafiltration also serves to free metabolic components that are otherwise bound to proteins as well as to reveal those hidden by more dominant resonances. The significant increase in β-hydroxybutyrate and acetate in severe with respect to mild OA SF is consistent with the hypothesis suggesting increased fatty acid utilization with OA state, since both substances are ketone byproducts of lipolysis. Alternately, these metabolites may be derived from the presence of an immunological infiltration; recent work has shown that lymphocyte metabolism is largely attributable to the oxidation of ketones.

Conclusion

In summary, we have shown that ¹H MRS can detect significant differences in the levels of endogenous low molecular weight metabolites in deproteinized SF between differing stages of OA.

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References
