GAG Content via Chemical Exchange Saturation Transfer: Assessing OA

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Introduction

Medical advances offer the hope that the detection of osteoarthritis (OA) at an early stage will allow one to intervene in a timely fashion to avoid severe manifestations of the disease. The early onset of degeneration is generally characterized by a loss of proteoglycans (PGs). A number of MRI techniques allow one to assay the loss of PGs and to deduce the stage of degeneration within cartilage tissue, based on ^{1}H MRI, as well as, ^{23}Na MRI [1-3].

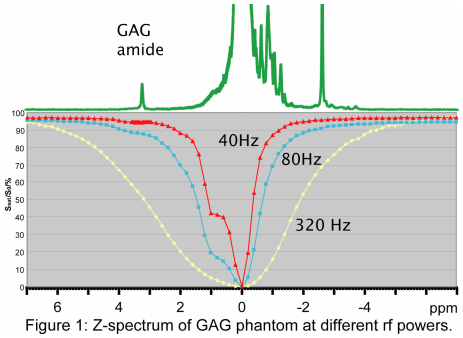

Figure 1: Z-spectrum of GAG phantom at different rf powers.

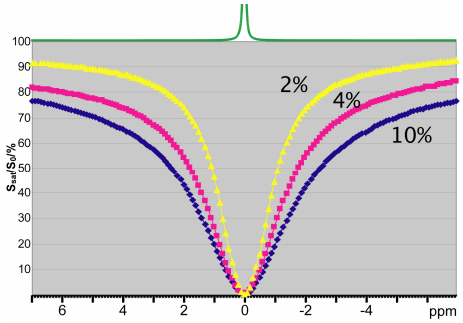

Figure 2: Evaluation of MT with different agarose conc.

The chemical exchange between the labile protons of low-concentration solutes and bulk water provides a novel sensitivity enhancement mechanism in MRI, first demonstrated by Balaban et al. (Chemical Exchange dependent Saturation Transfer – CEST) [4]. The representation of the CEST effect vs. the resonance offset is commonly termed z-spectrum. Based on a ^{1}H NMR spectroscopical study of GAG phantoms and degraded cartilage samples, we demonstrate a strong CEST effect that can be use to accurately measure the absolute glycosaminoglycan (GAG) concentration. The first images using CEST at 3T are underway.

Methods

The CEST effect is measured on a 500MHz Bruker Avance spectrometer equipped with a broadband inverse probe (BBI). Chondroitin sulfate phantoms with GAG concentrations of 150mM, 125mM, 100mM, 75mM, 50mM and 25mM at pH7.4 are used to evaluate the chemical exchange effect of PG. Agarose gel phantoms with concentrations 10%, 4% and 2% (w/w) are used to evaluate the magnetization transfer (MT) contribution to the CEST effect. Pieces of fresh bovine patellar cartilage were cut into a cylindrical shape of 6mm length and 5mm diameter to evaluate the cartilage tissue ex vivo. PG depletion was performed in a trypsin/PBS solution for 2 hours.

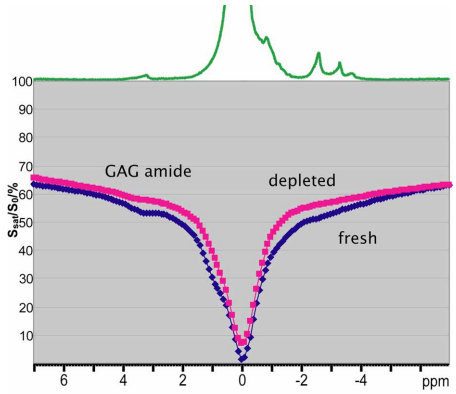

Figure 3: CEST of fresh and depleted cartilage.

Results & Discussion

In ^{1}H NMR spectroscopic analysis (HRMAS, TOCSY, HSQC) of PG we identified the amide proton as the strongest CEST agent, which has a one-to-one correspondence to GAG units in PG. Figure 1 shows the CEST z-spectra ([GAG]=150mM) with various irradiation power levels. Because of the mobility of PG in phantom/tissue, there is almost no MT effect from PG. Additional chemical exchange sites were identified as hydroxy groups. The amide proton demonstrated the strongest CEST effect at +3.3ppm, corresponding to the single amide proton per GAG unit.

The MT effect of the method was tested by the z-spectra of agarose gel at various concentrations (Fig. 2), which leads to increased broadening of the main dip in the spectrum as the agarose concentration increases.

The cartilage tissue samples were evaluated before and after depletion (Fig.3). The GAG depletion is clearly seen, as is some loss of the collagen matrix as evidenced by a decreased MT effect.

The CEST vs. [GAG] curves were generated with different irradiation power levels and show a consistent concentration dependence. The CEST effect demonstrates linear dependence on [GAG] (Fig.4). We display on the curve the GAG concentrations measured in two fresh cartilage samples, as well as the trypsin-depleted sample, which shows a lower concentration.

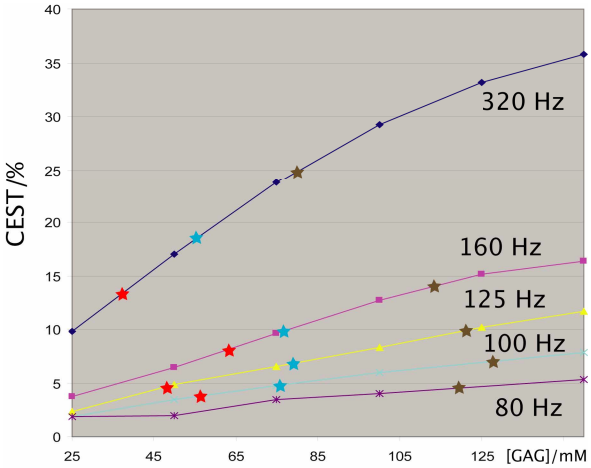

Figure 4: CEST vs. GAG conc at different rf powers. Stars mark the location of the cartilage measurements: Brown and cyan: two fresh cartilage samples; red: depleted cartilage sample.

Conclusion

The preliminary MRS/MRI studies confirm that the CEST effect from the amide proton of PG can be used to measure the absolute GAG concentration. In addition the change in MT effect can be used to quantitate the damage arising from the collagen matrix. Further quantification of [GAG] ex vivo and in vivo is underway on a 3T clinical MRI scanner. We expect the method will enhance the possibilities of detecting the early stages of OA.

Reference

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