gagCEST & NOE: Assessment of Glycosaminoglycan Concentration in Vivo

W. Ling[1], R. R. Regatte[2], G. Navon[1], and A. Jerschow[3]

[1]Tel Aviv University, Israel, [2]Medical School, New York University, New York, NY, United States, [3]New York University, New York, NY, United States

Introduction

GAG is essential for human musculoskeletal function, cell regulation, and spinal function. Proteoglycans (PGs), the functioning unit with high [GAG], play vital functions in diarthrodial joints and IVD (1). Thus, quantification of [GAG] in vivo is desirable for OA and disk degenerative diseases, which are both characterized by the loss of PGs in cartilaginous tissues. Based on our spectroscopic study (2) on cartilage, both amide proton (-NH, δ=+3.2ppm) and hydroxyl protons (-OH δ=+1.0~+1.9ppm) from GAG are exploited to be as CEST agents (3, 4).The annulus fibrosus and nucleus pulposus of IVD also demonstrates similar behavior as cartilage. Moreover, the contribution of the nuclear Overhauser effect (NOE) from slow motion of GAG was found to act like chemical exchange protons in z-spectroscopy, leading to additional diagnostic ability.

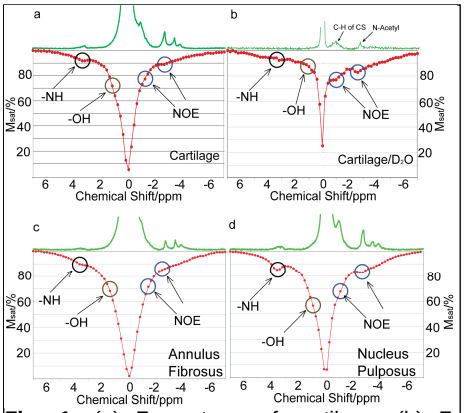

Fig. 1: (a) Z-spectrum of cartilage. (b) Z-spectrum of cartilage equilibrated in D_2O. (c), (d) Z-spectrum of IVD. The corresponding ^1H spectra are shown on the top of the z-spectra.

Materials and methods

NMR/MRI Sample preparation: The bovine cartilage samples were cut to into a 5 mm NMR tube. The 60 min trypsinization (0.2 mg/mL trypsin, Aldrich-Sigma) was performed a cartilage 60mins×3 times. In cartilage D_2O equilibration experiments, the fresh cartilage was immersed in PBS/D_2O for 24 hours. [^{23}Na] in fresh cartilage was calibrated. 1 human subject with occasional knee pain was under MR investigation.

NMR experiments: NMR Data were acquired at a 500 MHz Bruker Avance spectrometer equipped with a BBO probe. The temperature of the sample was stabilized at 310 K. The T_1 of bulk water was measured by saturation recovery. For the CEST experiments, continuous wave irradiation was used with irradiation power and duration varying according to the system of interest, followed by a 5° pulse. For the z-spectra, a total of 71 spectra were collected with 100 Hz shift in offset frequency per step. The saturation duration and power levels of the presaturation were as follows: For Figure 1: 10 s and 50 Hz; For Figure 2: 4 s and 250 Hz.

MRI experiments: The MRI experiments were performed on a 3.0 T clinical Siemens MR scanner. The CEST imaging sequence was performed with a train of ten 180° Gaussian pulses with pulse length 31 ms and average saturation power 35 Hz. Slice thickness = 3 mm; acquisition matrix = 256x128; FOV = 150 mm x 150 mm.

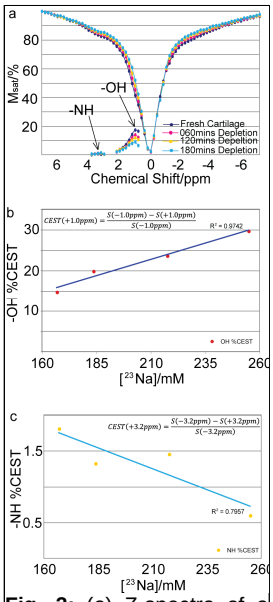

Fig. 2: (a) Z-spectra of a cartilage trysinization series. (b) -OH CEST vs. [^{23}Na], as assessed via ^{23}Na NMR spectroscopy. (c) -NH CEST vs. [^{23}Na], as assessed via ^{23}Na NMR spectroscopy.

Results and Discussion

The exchangeable protons of the PG molecules have been identified in a previous article (2). Figure 1a shows the z-spectrum from a piece of bovine cartilage: the chemical exchange sites are at δ=+3.2 and +1.0 ppm; additionally, two sites at δ=-2.6 ppm and -1.0 ppm are identified as NOE sites. These two sites correspond to the CH and N-acetyl residues in GAG, respectively (2). The NOE enhancement is expressed as $NOE_{water}=T_1\times\sigma$. The NOE sites were confirmed by Figure 1d, which shows the z-spectrum of cartilage equilibrated in D_2O. The presence of bulk D_2O significantly reduces the appearance of the dips at the chemical exchangeable sites, while it enhances the NOE sites as shown in Figures 1b. The enhancement can partly be attributed to the increase of T_1 in bulk D_2O compared to that in bulk H_2O: 2.1 s in native cartilage and 4.0 s in D_2O-equilibrated one. Figure 1c and 1d show z-spectra of annulus and nucleus region of IVD. Figure 2 shows the z-spectra acquired from a piece of a sequentially trypsinized cartilage. Figure 2b shows CEST(δ=+1.0 ppm) vs. [^{23}Na]. The linear relationship demonstrates that the -OH can be employed as a reliable CEST agent. In Figure 2c, however, -NH CEST(δ=+3.2 ppm) shows only about 1~2 % effect, much smaller than that from –OH, hence –NH CEST is less reliable. The high concentration (200~300 mM) and high chemical exchange rate ($k_{OH}\approx1000s^{-1}$) of –OH easily overrides the NOE contributions at δ=-1.0ppm, and results in an overall substantial CEST effect (15%~30%). Figure 3a shows in-vivo results of –OH CEST on a patellofemoral human knee joint, which shows a clear demarcation of a cartilage lesion on the medial facet. The regional variation of the GAG concentration is clearly demonstrated in Figure 3b. These results are consistent with the one from Figure 2b. Thus, the CEST difference image demonstrates its ability to detect localized GAG concentration distributions and hence the pathological state of cartilage in vivo.

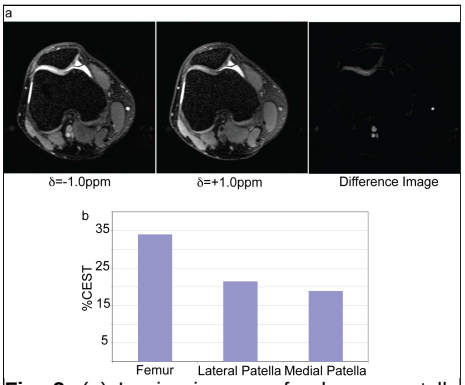

Fig. 3: (a) In vivo images of a human patella with irradiation at δ= -1.0 ppm, δ= +1.0 ppm, and the difference image. (b) The extracted CEST contrast.

Conclusion

We have demonstrated that –OH at δ=+1.0 ppm, among other labile protons, can be used to monitor GAG concentration in cartilage in vivo. The applicability of the method extends also to IVD diseases, as shown in Figs. 1c and 1d. The negative NOE observed in this work results from the interaction of water and non-exchangeable GAG protons with low mobility, which is likely to hold at lower magnetic fields, e.g. clinical scanners. Moreover, NOE is likely to contribute to the low efficiency of some –NH CEST based applications in vivo, but may lead to additional diagnostic methods for macromolecules, but may lead to additional diagnostic methods for macromolecules (5).

References

1. Roughley PJ (2006) *Eur. Cell Mater.* **12,** 92-101.
2. Ling W, Regatte RR, Schweitzer ME, & Jerschow A (2007) *NMR Biomed.* **20,** in press.
3. Ward KM, Aletras AH, & Balaban RS (2000) *J. Magn. Reson.* **143,** 79-87.
4. Zhou J & van Zijl PCM (2006) *Prog. NMR Spectr.* **48,** 109-136.
5. Hubbard PL, Närväinen J, Kauppinen RA, & Morris GA (2007) in *Proc. Intl. Soc. Mag. Reson. Med.* (ISMRM, Berlin, Germany), p. 3464.