Using the ionic x-ray contrast agent Hexabrix as a specific marker for cartilage glycosaminoglycan (GAG) content via chemical exchange saturation transfer imaging at 3 Tesla

Benjamin Schmitt[1], Toshiyuki Shiomi[2], Siegfried Trattnig[3], Monika Egerbacher[4], and Pavol Szomolanyi[3]

[1]*Centre for High-Field MR, Medical University of Vienna, Vienna, Austria,* [2]*Osaka University Graduate School of Medicine,* [3]*Medical University of Vienna,* [4]*University of Veterinary Medicine Vienna*

Introduction:

Glycosaminoglycans (GAG) are elementary components of cartilage tissue, and their loss is thought to be an early biomarker of osteoarthritis. The fixed charge density (FCD) of GAG is used for contrast agent based assessment of cartilage GAG content, such as in delayed gadolinium enhanced MRI of cartilage (dGEMRIC) [1]. The FCD can also be exploited for X-ray-based arthrography using ionic iodine-based contrast agents such as, e.g., Hexabrix® [2], which is a mixture of 39.3 % w/v ioxaglate meglumine and 19.6 % w/v ioxaglate sodium. Both molecules contain 4 amide protons and 2 hydroxyl protons, which may be subject to chemical exchange with surrounding bulk water molecules. This initial study was performed to evaluate if Hexabrix® can be used as a chemical exchange saturation transfer (CEST) agent [3] to assess cartilage GAG content with MRI at 3 Tesla.

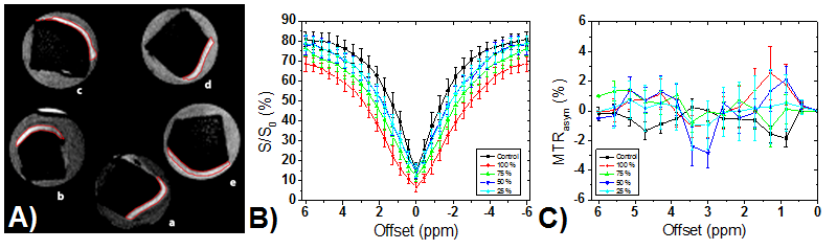

Fig. 1: A) CEST reference scan without RF presaturation showing the bone samples with healthy cartilage. Cartilage tissue could be segmented well (red margins). Sample **a** was the control without Hexabrix® (Hex), and samples **b**, **c**, **d**, and **e** were immersed in solution containing Hex in different concentrations (25 %, 50 %, 75 %, and 100 %). **B**) z-spectra and **C**) corresponding asymmetry analyses from segmented cartilage areas. Asymmetry curves do not show significant CEST effects, which can be attributed to exchangeable protons of Hex, and thus no accumulation of Hex can be observed in cartilage.

Materials & Methods:

Hexabrix® (Guerbet, France) 320 was used for MR experiments, and fresh (not previously frozen) porcine knees obtained from a local butcher were used as in-vitro cartilage samples. In a first experiment, the cartilage knees were divided into pieces, which were placed into plastic tubes and immersed in saline solutions with different concentrations (100 %, 75 %, 50 %, 25 % and one control) of Hexabrix® (Hex) for 24 h. In the second experiment, cartilage samples were degraded enzymatically for 1.5 h (2 samples) and 3 h (2 samples) using trypsine. After degradation, each 2 samples were placed in tubes and immersed in Hex solutions for 24 h. MR examinations were performed on a clinical 3 T System (Siemens Healthcare, Germany) using a standard 8-channel knee coil (InVivo, USA). CEST imaging was performed using 3D RF-spoiled gradient-echo (GRE) sequence with the following imaging parameters: T_E=3.88 ms, T_R=10.39 ms, 0.39x0.39x2.40 mm³, matrix=144x256x20, scan time 1 h 58 min (4 NEX). Selective RF presaturation was achieved using a series of 3 Gaussian RF-pulses with pulse duration τ_p =100 ms, interpulse delay τ_d =10 ms, and $B_{1\text{-CWAE}}$ = 2 µT. The saturation module was repeated every 48 phase encoding steps. The first image series was recorded as a reference without RF presaturation (S_0) and 27 series were acquired with RF presaturation Z-spectra from images were interpolated with a smoothing spline method and corrected for B_0 inhomogeneities on a pixel-by-pixel basis. From corrected z-spectra, the asymmetry of the magnetization transfer rate (MTR) was determined by $MTR_{asym}(\delta) = MTR(+\delta)-MTR(-\delta)$.

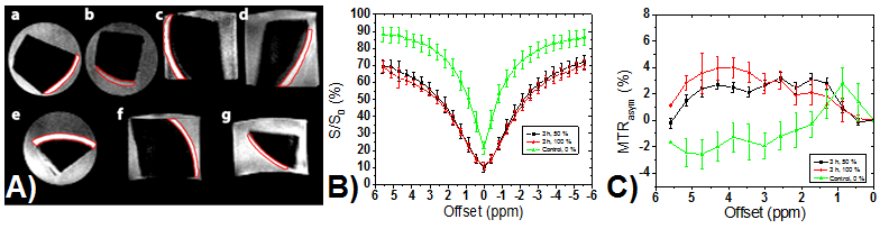

Fig. 2: A) CEST reference scan without RF presaturation showing the bone samples with trypsine degraded cartilage (**a,b**: 1.5 h degradation; **c,d**: 3 h; **e,f**: controls). Hex concentrations in the tubes were **a**: 50 %, **b**: 100 %, **c**: 50 %, **d**: 100%, **e**: control, **f**: 50 %, **g**: 100 %. **B**) z-spectra from segmented cartilage in samples **c, d** and **e** showing similarly reduced dynamic range for trypsine treated cartilage compared to control. For reasons of readability curves from remaining samples are not shown. **C**) Corresponding asymmetry analyses. Asymmetry curves show a broad increase between from δ = 3.5 to 4.8 ppm, which could indicate CEST effects due to Hex accumulation in cartilage.

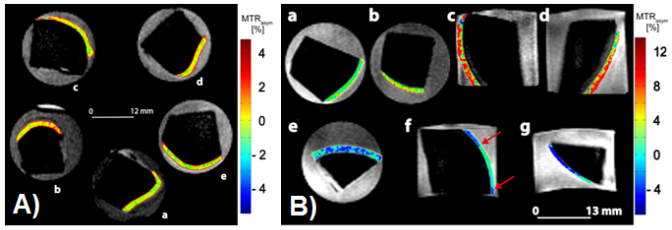

Fig. 3: Overlay of CEST signal intensities (pixel MTR_{asym} value at δ = 4.25 ppm) in cartilage on grayscale CEST reference image. **A**) Untreated samples in the same order as in Fig. 1A. **B**) Samples treated with trypsine (**a-d**) and controls (**e-g**) in the same order as in Fig. 2A. The treated cartilage shows clearly elevated signals compared to controls. CEST signals of cartilage after 3 h depletion (**c,d**) appear to be higher than the values of cartilage after 1.5 h, which could indicate stronger accumulation of Hex in **c** and **d** due to lower GAG content. Interestingly, control cartilage in **f** exhibits focal signal increase (arrows), which could be a sign of mechanical damage of cartilage from, e.g., cutting of the bone samples.

Results:

Spatial resolution of datasets was sufficient to delineate cartilage from bone tissue in the examined samples (Fig. 1A, 2A). In healthy cartilage, no apparent increase of MTR_{asym} at δ = 4.25 ppm that can clearly be related to Hex accumulation in cartilage was observed (Fig. 1C, 3A). Instead, the MTR_{asym} curves showed similar patterns as reported for healthy cartilage tissue in CEST experiments with endogenous contrast. The average MTR_{asym} values at δ = 4.25 ppm ranged between 0.54 and 1.72 % in samples with different Hex concentrations and were –0.88±0.63 % (mean ± SD) in control (Fig. 1C). GAG content was strongly reduced by trypsine treatment, which was confirmed by histologic staining. Depleted cartilage showed clear increases in MTR_{asym} values at δ = 4.25 ppm compared to healthy cartilage (Fig. 2C, 3B). After 3 h degradation, MTR_{asym} was 2.68±0.32 % (50 % Hex), 3.94 ± 0.87 % (100 % Hex), and after 1.5 h of degradation, 2.01±0.18 % (50 % Hex), 2.24±0.31 % (100 % Hex). Additionally, asymmetry curves showed clear signal increases between δ = 3.5 to 4.8 ppm, which corresponds to the resonance frequency of the amide protons of Hex.

Discussion and Conclusion:

We found specific increases of MTR asymmetry values in enzymatically degraded cartilage after being immersed in Hex. Since a reduced GAG content in these samples was confirmed by histologic staining, we attribute the observed increases to an accumulation of Hex in the cartilage as this is in good agreement with theory, which yields that the negatively charged contrast agent can only diffuse and accumulate in cartilage if FCD is reduced compared to healthy cartilage. This is the case when GAG are enzymatically degraded. These initial experiments therefore suggest that Hex is a potential CEST agent, which may be used for assessment of cartilage GAG content. The technique could furthermore be an alternative to Gd-based MR techniques used for this purpose, such as, e.g., dGEMRIC. However, further evaluations of pharmacokinetics as well as in vivo experiments are needed in order to confirm these initial in vitro results, and gain knowledge about possible specificity and sensitivity of the method.

References: [1] Bashir A *et al*. Radiology 1997;205. [2] Palmer AW *et al*. PNAS 2006; 103(51). [3] Sherry AD & Woods M. Annu Rev Biomed Eng 2008; 10.