Assessment of Porcine Intervertebral Disc Specimen pH via Chemical Exchange Saturation Transfer (CEST) MRI

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Introduction: Intervertebral disc (IVD) degeneration is considered one of the underlying factors of low back pain [1]. The IVD is highly avascular. Its nutrients must diffuse from peripheral capillaries and its energy is mainly generated by anaerobic glycolysis [2]. When the IVD cells are metabolically taxed, lactate (Lac) is accumulating, the pH drops and this has been shown to cause a number of metabolic changes to the cell of the IVD [3]. Studies have linked low pH and loss of glycosaminoglycan (gag) in patients IVDs with discogenic back pain [4]. The accumulation of Lac and a decrease in pH seem to be initiating steps in disc degeneration and discogenic back pain. Therefore MRI methods to measure pH in IVD would be valuable in research and clinical settings, gagCEST, which detects the amount of gag by off-resonance irradiation of the exchangeable hydroxyl protons [5], may also be the sensitive to pH, due to the sensitivity -OH to pH [6]. In the first part of the present study two questions are investigated: (i) whether gagCEST is pH dependent and (ii) whether gagCEST can be used to detect pH changes in an IVD. It has also been shown previously that the DIACEST contrast agent Iopromide could be used to measure the extracellular pH in tumors by detecting two pH dependent amide groups using CEST [7]. Therefore the pH measurement using Iopromide is independent of the local concentration of the molecule. In the second part of the present study CEST MRI is used to investigate if pH changes in the IVD are detectable using Iopromide.

Material and Methods: Phantoms: A chondroitin sulfate solution (Sigma-Aldrich, St. Louis, MO) was diluted with H2O to 200 mM and separated in eight 5nm diameter NMR tubes, which were titrated with NaOH/HCl to obtain a range of pH values: 5.66, 5.96, 6.10, 6.49, 6.76, 7.07, 7.5 and 7.86. In a second set of seven 5nm NMR tubes a 200mM solution of Iopromide (Ultravist 370, Bayer Healthcare) was titrated to a range of pH values of: 5.96, 6.18, 6.40, 6.70, 6.88, 7.12 and 7.48. The pH values were measured using a pH meter. Specimens: Porcine lumbar spine samples were obtained from a U.S. Department of Agriculture-approved slaughterhouse (Baigio Artisan Meats, Oakland, CA) 5-6h after slaughter from 2- to 5-month-old piglets. The pH of the porcine IVDs was manipulated using a 1M-lactate solution, which was injected into the IVDs. MRI: Imaging was performed on 7T Agilent horizontal MR scanner equipped with a 400mT/m gradient system using a 38mm diameter 1H quadrature birdcage coil. A pulsed CEST preparation module in combination with a single slice turbo spin-echo imaging sequence (TR=3s, TE=35.5ms, FOV=40x40mm², matrix=64x64) was used for Z-spectra acquisition. The CEST preparation module consisted of 30 Gaussian pulses (pulse-length=100ms, bandwidth=25Hz (FWHM)) and B1=0.75μT. Z-spectra were acquired over a 1kHz to 1kHz frequency range. For B1-correction WASSR spectra were acquired and the asymmetry of the magnetization transfer rate (MTR asym) was calculated [8].

Results: Fig. 1a shows the MTR asym plots from the different pH chondroitin sulfate phantoms. An intensity- and shape-based pH dependence of hydroxyl group can be observed. Fig. 1b shows the T2-weighted reference image of the phantom, Fig. 1c the MTR asym map at an offset of 220Hz (from 0.75ppm from the water resonance). The intensity of MTR asym at this offset is pH dependent, Fig. 1d displays the integral of the MTR asym from 0.5 to 1.5ppm offset and Fig. 1e the MTR asym at the 0.75ppm offset as a function of pH.

Discussion: gagCEST, which uses the irradiation of the hydroxyl protons of gag, could be a technique to detect pH changes in IVD in vivo (if the gag concentration is known). The shown pH dependence can help to understand gagCEST data from IVDs. Iopromide is a promising molecule to measure pH in IVDs (independent from the local concentration), but must be applied directly (invasively) into the IVDs.

References: