Demonstration of the pre-clinical efficacy of Aggrecanase Selective Inhibitor by T1ρ MRI

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Objective
The objective of this study was to demonstrate the effectiveness of T1ρ MRI as a pre-clinical tool in drug development. Specifically, we tested whether T1ρ would measure modulation of IL-1β induced degeneration of ex vivo cartilage by an Aggrecanase Selective Inhibitor (ASI).

Background
Osteoarthritis (OA) is the most common degenerative joint disease, yet currently there is no quantitative and noninvasive method to evaluate the efficacy of potential drugs that may alleviate OA and prevent cartilage degeneration. Previously, we have shown that T1ρ relaxation is sensitive to models of arthritis both in vivo and ex vivo (1, 2). T1ρ MRI is correlated with GAG loss in early stages of cartilage degeneration (3). One key modulator in the disruption of GAG matrix is interleukin-1β (IL-1β), a cytokine produced by inflammatory cells and chondrocytes (4). In this study, using T1ρ MRI we measured changes in ex vivo cartilage induced by IL-1β that are blocked by an ASI. The T1ρ MRI results were validated with the standard 1, 9-dimethylmethylene blue (DMMB) (5) proteoglycan assay.

Materials and Methods
The Institutional Animal Care and Use Committee (IACUC) approved these studies. Young calf patellas obtained from a local slaughter house were cored to 8 mm diameter, 2 mm thick cylinders from the cartilage and underlying bone of the patella. Individual cores were then incubated in 1 ml DMEM media with glucose (4.5 mg/mL), 1x pen-strep-ampphotericin B, 1x Insulin-Transferrin-Selenium-G, and vitamin-C (12 mg/mL) for 5 days in a 48 well plate to allow for equilibration. After the second day of equilibration, lactate assays (Biovision, Catalog#K607-100) were performed on the culture media to assess cell viability. Only cores that produced lactate above 75 nmol/well (100 µL) were taken into the study. After five days of equilibration, the viable cores were each placed in 400 µL of media and categorized into 4 groups of 5 each: control, IL-1β, IL-1β with an ASI, and control with ASI. Culture media was refreshed each day. DMMB assay was performed on the media each day by analyzing the absorbance at 525 nm to assess GAG release, an indicator of cartilage matrix turnover. Cores were imaged on the 3rd and 6th days. All MR imaging was performed on a 3T clinical Siemens Trio scanner interfaced with a custom-built 2.8 cm diameter mu solenoid coil. A T1ρ map for each group was obtained. A T1ρ imaging sequence was repeated 5 times for each condition with varying spin lock duration. Sequence parameters were: spin lock amplitude 500 Hz, spin lock durations 12 ms, 24 ms, 36 ms, 48 ms, and 60 ms, FOV 31x31 mm, slice thickness 1 mm, Matrix size 256x256, TR/TE 3000/17 ms, Turbo factor 5, scan time per map 10 min 45 sec. The data was processed using a previously published approach (3).

Results
Figure 1 shows the representative GAG release in the media by DMMB assay. Figure 2 shows representative T1ρ maps for each group. Figure 3 presents the pooled T1ρ values (n=20-25 per group) taken from region of interest on the third day of treatment of the four groups. The T1ρ values of the cartilage show that treatment with IL-1β increases T1ρ values, while the cores treated with IL-1β plus ASI and ASI alone have T1ρ values comparable to the control group. Figure 4 shows corresponding Alcian blue-PAS stained histology of a member of each of the four groups. Intensity of blue represents the presence of GAG.

Conclusions
Based on the T1ρ MRI, lactate, DMMB assay, and histology data, we draw the following conclusions from the study:

- Compared to the control group, T1ρ was increased substantially in the cartilage cores treated with IL-1β. T1ρ remained almost unchanged in the group treated with IL-1β and ASI, demonstrating the activity of the selective ASI to block IL-1β induced matrix degradation. The low level of GAG released in the media as measured by the DMMB assay supports this finding by T1ρ. T1ρ also remained almost unchanged (compared to control) in the group treated with the ASI alone. This indicates that the drug (at the dose levels used) alone would not cause any action that leads to GAG loss. In groups 3 and 4 (Figure 2) there is a slight decrease in T1ρ and GAG release (Figure 1) compared to control. A similar trend exists in the histology sections. This may be hypothesized to be due to the action of the drug that blocks the auto degradation of the matrix and associated GAG loss. This study demonstrates that the T1ρ MRI can be used to study the efficacy of potential therapies ex vivo.

References: