Demonstration of the pre-clinical efficacy of Aggrecanase Selective Inhibitor by T1p MRI

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Objective

The objective of this study was to demonstrate the effectiveness of $T_{1\rho}$ MRI as a pre-clinical tool in drug development. Specifically, we tested whether $T_{1\rho}$ 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 $T_{1\rho}$ relaxation is sensitive to models of arthritis both *in vivo* and *ex vivo* (1, 2). $T_{1\rho}$ 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 $T_{1\rho}$ MRI we measured changes in *ex vivo* cartilage induced by IL-1β that are blocked by an ASI. The $T_{1\rho}$ 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 8mm diameter, 2mm thick cylinders from the cartilage and underlying bone of the patella. Individual cores were then incubated in 1mL DMEM media with glucose (4.5mg/ml), 1X pen-strep+amphotericin B, 1X Insulin-Transferrin-Selenium-G, and vitamin-C (12mg/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 75nmol/well (100µl) were taken into the study. After five days of equilibration, the viable cores were each placed in 400µL 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 2.8cm diameter multi-turn solenoid coil. A $T_{1\rho}$ pre-encoded Turbo Spin Echo sequence was repeated 5 times for each condition with varying spin lock duration. Sequence parameters were: spin lock amplitude 500Hz, spin lock durations 12ms, 24ms, 36ms, 48ms, and 60ms, FOV 31x31mm, slice thickness 1mm, Matrix size 256x256, TR/TE 3000/17ms, 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 $T_{1\rho}$ maps for each group. **Figure 3** presents the pooled $T_{1\rho}$ values (n=20-25 per group) taken from region of interest on the third day of treatment of the four groups. The $T_{1\rho}$ values of the cartilage show that treatment with IL-1β increases $T_{1\rho}$ values, while the cores treated with IL-1β plus ASI and ASI alone have $T_{1\rho}$ 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.

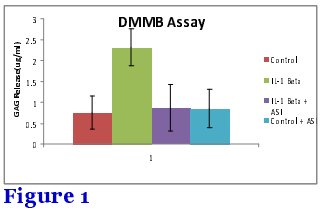

Figure 1

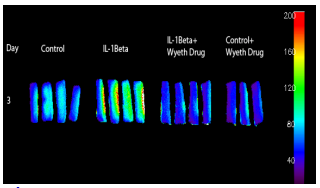

Figure 2

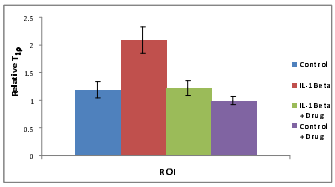

Figure 3

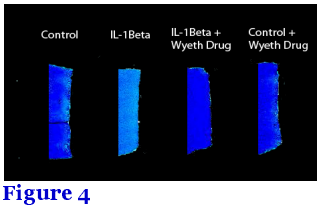

Figure 4

Conclusions

Based on the $T_{1\rho}$ MRI, lactate, DMMB assay, and histology data, we draw the following conclusions from the study:

Compared to the control group, $T_{1\rho}$ was increased substantially in the cartilage cores treated with IL-1β. $T_{1\rho}$ 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 $T_{1\rho}$. $T_{1\rho}$ 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 $T_{1\rho}$ 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 $T_{1\rho}$ MRI can be used to study the efficacy of potential therapies *ex vivo*.

References:

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