Magnetic Resonance evaluation of earlier intervertebral disc degeneration (IVD): in vivo and in vitro use of T1 rho sequences

R. Del Vescovo

S. Battisti, L. Stellato, F. Martina, V. Denaro, B. Beomonte Zobel

University of Rome “Campus Bio-Medico”
Dept. of Diagnostic Imaging, Chairman Prof. B. Beomonte Zobel
Propose

To show the use of T1rho-weighted Magnetic Resonance Imaging (MRI) for the assessment of intervertebral disc degeneration (IDD) and proteoglycan content in the bovine intervertebral disc explant model.

“Magnetic Resonance (MR) T1rho relaxation time is associated with loss of macromolecules in the matrix of the intervertebral disc may be an initiating factor in degenerative disc disease”

METHODS AND MATERIALS:

-Phantoms were created with different concentration of MnCl₂ to calibrate T2 relaxation value and T1rho value.

-Intact bovine tails were imaged on a clinical 1.5 Tesla MR scanner. T1- and T2-weighted images and T1rho-weighted images were obtained in basal condition. The bovine NPs were injected with collagenase enzyme or buffer solution. The tails were incubated at 37°C for 12 hours. After the collagenase’s digestion, the tails were analyzed by the MR scanner to obtain T1-T2- and T1rho-weighted images. The proteoglycan’s disc content was also performed to obtain a correlation between T1rho value and PG.
Bovine Tail

- Bovine caudal discs have been developed as a suitable model for many in vitro studies of the intervertebral disc, having similar physico-chemical properties to the human disc.

- Bovine tails were obtained from animals aged 24 months at a local abattoir within 1 hour of death. Under sterile condition we cleared the tail, fat tissue and some muscle were removed from the upper tail without trimming the tail to the bone.

- Six motion segments were injected for induction of degeneration of NP with the current protocol on cranio-caudal direction
**DATA PROCESS: T1 Rho Post Collagenase**

<table>
<thead>
<tr>
<th>Discs number</th>
<th>TSL 75ms</th>
<th>TSL 60ms</th>
<th>TSL 45ms</th>
<th>TSL 30ms</th>
<th>TSL 15ms</th>
<th>T1RHO VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>62</td>
<td>80</td>
<td>91</td>
<td>113</td>
<td>76,92</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>59</td>
<td>63</td>
<td>101</td>
<td>102</td>
<td>71,42</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>69</td>
<td>97</td>
<td>135</td>
<td>138</td>
<td>47,61</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>35</td>
<td>28</td>
<td>58</td>
<td>83</td>
<td>58,82</td>
</tr>
</tbody>
</table>

**Collagenase Protocol**
Collagenase discs’s injection (cranio-caudal)
1\textsuperscript{st} disc $\rightarrow 0.75 \mu l$
2\textsuperscript{nd} disc $\rightarrow 1.25 \mu l$
3\textsuperscript{th} disc $\rightarrow 2.5 \mu l$
4\textsuperscript{th} disc $\rightarrow 5 \mu l$
Statistical Analysis using mean T1rho value

$t = 3.8232$

$P = 0.0087$

Result

![T1rho value: Pre Vs Post Collagenase](chart.png)
To confirm the degradation enzyme-induced axial images were obtained, where better shown the different regions of the disc: NP (in the center hyperintence) and AF (the anular periferical region)
The discs were dissected and NP and AF were separated for biochemical analyses. Linear regressions of T1rho versus water and PG contents were obtained.

The nucleus pulposus tissue were used to determine water and sulfated-glycosaminoglycan content.

The wet weight of each tissue sample was first recorded in triplicate. Samples were then lyophilized, dry weight was recorded in triplicate, and the percentage water content was calculated.

To determine sulfated-glycosaminoglycan content, 5-mL aliquots were pipetted into a ninety-six-well plate and were analyzed with a microplate reader and the 1,9-dimethylmethylene blue assay. The sulfated-glycosaminoglycan content was normalized to dry weight and wet weight.

A positive linear correlation was observed between the T1r relaxation time on the images of the nucleuspulposus and glycosaminoglycan content per dry weight (r = 0.69), glycosaminoglycan per wet weight (r = 0.49), and water content (r = 0.53).
Correlation between T1rho relaxation time and sulfated-glycosaminoglycan content (s-GAG) per dry weight (A) and between T1rho relaxation time and water content (B)
Results

Collagenase degradation of the NP significantly decreased the relaxation times and the magnetization transfer ratio. In discs injected with collagenase, reduction of proteoglycan content is obtained and linear-regression between PG content and T1rho value is shown. Instead disc injected with buffer does not present any significant proteoglycan content reduction. These results are also confirmed by extraction analysis.
Conclusions

- T1rho value is strongly with the concentration of proteoglycan in the NP, instead T2 relaxation times correlate weakly with PG content.
- Our results demonstrate that early changes in the NP matrix proteins can be assess with T1rho-weighted imaging.
- The ability to identify and quantify these early biochemical changes will provide a better understanding of the patho-physiology of disc degeneration and facilitate the study of interventions that aim to halt or reverse the degenerative process.
Asymptomatic young male (19 y.o.). The intervertebral disc L5-S1 showed a low T1rho value 76.9ms, it is correlated with a early loss of PG that is not evident with T2 weighted images.