H-1 DOUBLE-QUANTUM-FILTERED NMR IMAGING CAN DEPICT MATURATION AND ORDERING OF COLLAGEN FIBERS IN REGENERATING TENDON

INTRODUCTION:

Conventional magnetic resonance imaging (MRI) techniques depict intact tendon tissue with negative contrast to the surrounding soft tissues with higher signal intensity. This is because most of the water molecules in the tendon interact with collagen fibers, and thus have short transverse relaxation time (T2). The collagen fibers in the tendon are highly oriented in its longitudinal direction, inducing anisotropy in magnetic relaxation of the bound water molecules as well. We have demonstrated that maturation and ordering of the collagen fibers in the regenerating Achilles tendon can be evaluated through the analysis of T2 relaxation anisotropy (2).

Double quantum filtered (DQF) spectroscopy is a technique sensitive to the anisotropic motion of quadrupolar nuclei. Recently, it is reported that the residual dipolar splitting of H2O was detected by using the DQF nuclear magnetic resonance (NMR) technique (3). The application of this technique to the MRI enabled the visualization of the bound and ordered water molecules in the normal tendon with positive contrast (4). Here we analyzed the 1H DQF NMR signal from the regenerating tendon to show its possible application as an MR imaging technique in the monitoring of the structural recovery of the tissue.

METHODS:

Twenty-three male Japanese white rabbits (mean weight 2.5 kg) were used. Six Achilles tendons from three rabbits were used as controls. The twenty rabbits were anesthetized with sodium pentobarbital (25 mg/kg body weight) and their right Achilles tendons were sharply cut transversely 1 cm above their calcaneal points. At 3, 9, 13, and 18 weeks after the Achilles tenotomy, the regenerating tendon was dissected en bloc with the surrounding tissues, and was placed in an NMR sample tube and the longitudinal axis of the tendon was aligned parallel to the static magnetic field. 1H DQF spectra were obtained using the following pulse sequence:

\[ \tau \rightarrow 90^\circ - t_2 = 1.8^\circ - 2\tau - 90^\circ \rightarrow 90^\circ (aquisition) \]

where \( \tau \) is the creation time and \( t_2 \) is the evolution time of the DQ coherences. We measured the relative intensity, from which we got the peak intensity and the creation time dependency. The 1H DQF images were obtained using an established technique (4) with the slice selection. All the NMR examinations were done using an NMR spectrometer (field strength 7.05 T; Bruker, Germany) at 25°C.

RESULTS AND DISCUSSIONS:

The 1H DQF NMR technique is sensitive to the anisotropic motion of the water molecules. The ordering of the collagen fibers can be evaluated indirectly through the measurement of the 1H DQF signal of the water, which is bound to the fibers. The mean 1H DQF NMR signal intensity of the control Achilles tendons was 11.76 ± 0.99 %, and the residual dipolar coupling of the control Achilles tendons was 686.2 ± 59.1 Hz. With regard to the regenerating tendons, the 1H DQF NMR signal intensity became higher, and the residual dipolar coupling became stronger. These results reflect an increase in both the number and the order of the collagen fibers in the regenerating tendon.

The control tendons were imaged with a high signal in the 1H DQF NMR image when we adjusted the \( \tau \) from 400 to 600 \( \mu \)s, however no signal was visible when we adjusted it longer than 1 ms. At 3 weeks after the tenotomy, no visible signal from the regenerating tendon was observed in the 1H DQF NMR image under the any \( \tau \) condition. This is due to too small a fraction of the bound and ordered water in the regenerating tendon at 3 weeks after the tenotomy. In accordance with an increase in the fraction of the bound and ordered water in the regenerating tendon during the maturation, the residual dipolar coupling of the regenerating tendon became bigger (260.0 Hz at 9 weeks and 413.8 Hz at 18 weeks after the tenotomy). Hence, the 1H DQF NMR imaging with a \( \tau \) of 0.4-2.0 ms clearly showed the regenerating tendon at 9 weeks after the tenotomy with positive contrast (Fig. 1). At this time, a positive signal was obtained only from the regenerating tendon and not from the surrounding intact tendons, which demonstrated a clear distinction between them. In contrast, such a distinction was impossible using gradient echo (GE) images because both the surrounding and regenerating tendons emitted very low signal.

In conclusion, the 1H DQF MRI is a useful non-invasive technique to evaluate the maturation and the ordering of the collagen fibers in the regenerating tendon, which have never previously been achieved through other diagnostic modalities.

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REFERENCES:

2. Takamiya H. et al. Trans. ORS, 24: 1078, 1999

Table 1: Residual dipolar coupling and peak intensity of 1H DQF NMR signal during the maturation of the regenerating tendon (mean ± SEM, n=5).

<table>
<thead>
<tr>
<th>Time</th>
<th>Residual Dipolar Coupling (Hz)</th>
<th>Peak Intensity (%)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>686.2 ± 59.1</td>
<td>11.76 ± 0.99</td>
</tr>
<tr>
<td>3 weeks</td>
<td>128.8 ± 7.7</td>
<td>0.41 ± 0.12</td>
</tr>
<tr>
<td>9 weeks</td>
<td>260.0 ± 24.3</td>
<td>3.14 ± 0.82</td>
</tr>
<tr>
<td>13 weeks</td>
<td>366.0 ± 33.4</td>
<td>4.29 ± 0.44</td>
</tr>
<tr>
<td>18 weeks</td>
<td>413.8 ± 29.2</td>
<td>7.68 ± 0.65</td>
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Fig. 1: Axial images of the regenerating tendon at 9 weeks after the tenotomy using GE pulse sequence (a). 1H DQF pulse sequence at \( \tau = 0.4 \) ms (b), 1.0 ms (c), and 2.0 ms (d). At \( \tau = 1.0 \) ms, regenerating Achilles tendon is clearly distinguished from the surrounding intact tendons. (1):Intact flexor digitorum superficialis tendon. (2):Regenerating Achilles tendon. (3):Intact flexor digitorum profundus tendon. (4):Tibia bone.

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