In Vivo Determination of the Anisotropic Diffusion of Water and the T1 and T2 Times in the Rabbit Lens by High-Resolution Magnetic Resonance Imaging

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Purpose. Several magnetic resonance imaging (MRI) "tools" for ophthalmologic research have recently been developed in this laboratory, including improved gradient and radiofrequency coils and pulse sequences for high-resolution and diffusion imaging (100-μm resolution).

Methods. These tools have been applied to the in vivo measurement of the relaxation parameters (T1 and T2) and the water diffusion coefficients (Dx and Dy) in the rabbit eye lens, both normal and cataractous. Maps of these parameters in the lens have been computer generated.

Results. In the normal lens, water diffusion is highly anisotropic and tends to be parallel to the surface. In the trauma-induced cataractous lens, an increase in spin-spin relaxation times (T2) consistent with edema and alteration of diffusion patterns was observed in a study conducted 2 weeks postsurgery. A partial reversal was observed 6 weeks postsurgery. The histologic data on the enucleated lens at 6 weeks showed a loss of normal lens architecture. Images are shown that display other small structures of the anterior segment with great clarity.

Conclusions. An extension of this work, now underway, is the study of the formation of various types of cataract in animal models. It is hypothesized that these methods can be extended to humans as a quantitative alternative for the assessment of cataracts. Invest Ophthalmol Vis Sci. 1993;34:2151-2158.

Since its introduction early last decade, magnetic resonance imaging (MRI) has been shown to be clinically useful in ophthalmology1-5 because of its multiplanar capability and prominent soft-tissue contrast. In an attempt to understand the mechanism of cataract formation, MRI has been used on the excised lens to study the structural properties and changes in different osmotic cataract models.6-9 However, in vivo studies of the lens have been hampered by low resolution and a poor signal-to-noise ratio (SNR), using conventional low- and midfield MRI scanners. Recently, we have obtained high-resolution orbital images of anesthetized rabbits with an improved surface coil and software.6 In the current study, further improvements in resolution and SNR were achieved by using a three-axis local-gradient coil of our own design in a 1.5-Tesla General Electric Signa scanner (G. E. Medical Systems, Waukesha, WI).7

The anatomic detail and contrast between tissues in a conventional MRI image depends on three physical properties: the proton density, spin–lattice relaxation time (T1), and spin–spin relaxation time (T2). Tissue T1 and T2 data are useful in structural characterization and image-quality optimization. The lens is a highly ordered collection of long, thin fibers whose
main cytoplasmic content is protein crystallins. This highly ordered matrix and low hydration state is responsible for the short T1 and unusually short T2 of the lens.\(^6\)\(^-\)\(^10\) A conventional MRI apparatus is limited. Using conventional gradients (1 Gauss/cm) and conventional pulse sequences, the echo time (TE) is limited to values longer than approximately 20 msec for small fields of view. For optimum SNR, the TE should be less than the T2, and difficulty in satisfying this condition has limited the application of MRI to the lens. Employing the local-gradient coil and modified software, we have obtained good images from which T1 and T2 information was extracted using a spin-echo sequence with a short TE to overcome the problem of fast T2 decay.

Diffusion coefficients in different regions of the lens have been studied in the past using various methods.\(^11\)\(^-\)\(^12\) MRI is the only in vivo technique available currently for measuring proton diffusion. The proton translational diffusion coefficient, in addition to T1 and T2, can be another indicator of molecular organization in the lens. Using the local-gradient coil with a pulse sequence first proposed by Stejskal and Tanner\(^15\) and later modified for MRI by Le Bihan et al,\(^14\) proton diffusion coefficient studies have been performed on the lens and are reported here. Diffusion is appropriately described by a tensor. Diffusion coefficients in the x and y directions have been determined in the current study at every point (or voxel) in space. The anisotropy of diffusion is of particular interest.

The development of cataract is associated with known molecular and structural changes in the lens. Characterization of structural properties of lenses in vitro have been made using proton magnetic resonance techniques.\(^11\)\(^-\)\(^15\) The observed relaxation changes, especially T2, were the result of an increased hydration fraction and disruption of the lattice structure in the cataractous lens. It is also natural to expect changes in proton diffusion patterns in different regions of the lens. Using a microsurgically induced traumatic cataract model, we followed structural changes in T1, T2, and the diffusion pattern of the lens at different stages postsurgery. Our results show anisotropic proton diffusion and determine T1 and T2 relaxation times in various regions of both normal and cataractous lenses.

**MATERIALS AND METHODS**

**Experimental System**

All MRI experiments were done in a 10-cm diameter x, y, z local-gradient coil of our own construction designed using the method of conjugate gradient decent.\(^16\) A 7.5-cm internal diameter saddle coil of low Q was inserted into the gradient coil for radiofrequency transmission. A 2.5-cm diameter two-turn surface coil with passive decoupling\(^17\) was mounted inside the transmission coil. The assembly was used in the General Electric 1.5-Tesla Signa scanner at the Milwaukee County Medical Center with pulse programs of our own design.

**Imaging Methods and Postprocessing**

For the acquisition of T1 and T2 information, conventional spin–echo sequences were used. To achieve small fields of view and a short TE simultaneously, the bandwidth of the data collection was increased from 16 to 32 kHz, decreasing the data acquisition time and allowing a TE of 8 msec to be used. At 4 X 4 cm and 3 X 3 cm fields of view, 4- and 5.3-Gauss/cm gradient strengths were required, respectively. Four echoes (6, 16, 24, and 32 msec TE) for each repetition time (TR; 500, 1000, and 2000 msec) were collected in each scan. The other MRI parameters were: slice thickness, 3 mm; no phase wrap; number of excitations, 1; and image matrix, 256 X 256.

Diffusion images were obtained as described earlier.\(^14\) The parameters were: TE, 35 msec; TR, 500 msec; no phase wrap; number of excitations, 2; diffusion weighting gradient pulses, 10.0-msec duration; and 8 Gauss/cm amplitude, giving a b factor of 682 sec/mm\(^2\).

A fast algorithm for computing pure T1, T2, and diffusion coefficient maps from orbital images was employed.\(^18\) It uses a vector projection method. The values of a given pixel in the T1, T2, or diffusion image series represent components of an n-dimensional vector, where n is the number of images in the series. This vector is projected onto normalized sets of vectors representing different relaxation times or diffusion coefficients. The highest scalar product gives the relaxation time at a tested pixel. T1 maps were reconstructed from images with 8 msec TE and TR values of 300, 1000, and 2000 msec. T2 maps were reconstructed from four images obtained with multiple echoes and a 2000-msec TR. Diffusion coefficient maps were reconstructed from pairs of images: one with no diffusion weighting and one with a diffusion weighting gradient applied. Values of T1, T2, and the diffusion coefficient from selected regions of interest (greater than 50 voxels for each ROI) in the lens were determined from the reconstructed maps.

**ANIMALS**

All animals used in this study were treated in accordance with institutional guidelines and the ARVO Resolution on the Use of Animals in Research. Two-month-old Dutch rabbits (n = 3), weight range 1–2.5 kg, were anesthetized with a mixture of ketamine (45 mg/kg) and xylazine (5 mg/kg) intramuscularly. The anesthetized rabbits were placed inside the local-gra-
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dient coil with their heads resting inside the transmission coil and the surface coil on top of one eye without physical compression. A booster dose (50% of the initial dose) of anesthetic was administered every 40–50 min to ensure immobilization of the rabbit and acquisition of images at identical anatomic locations.

Before the surgery to induce a traumatic cataract, the rabbit was anesthetized also with ketamine and xylazine, and the eye was pharmacologically dilated. Using standard microsurgical techniques, a conjunctival peritomy was performed at the 9 o’clock position of the right eye. A 27-gauge needle was passed 2 mm posterior to the limbus, and the posterior capsule of the lens was injured. The posterior chamber was irrigated with balanced salt solution, and the needle was removed. Ophthalmic examinations and MRI experiments were performed 2 weeks and 6 weeks after the surgery. The rabbit was then killed, and the enucleated lens was fixed in a 10% neutral buffered formaldehyde solution before standard histopathologic preparation.

RESULTS

Figure 1 shows axial images of a normal right eye and the cataractous left orbit at 2 and 6 weeks, with the plane of section positioned through the center of the lens. These proton density-weighted images exhibited a high degree of contrast among anterior segment microstructures, such as tarsal glands, cornea, iris, ciliary process, choroid, sclera, and lens. In the normal lens, the short TE used, 8 msec, allowed exceptional SNR in all regions of the lens. Two weeks after the surgical procedure, a white opacified lens was present. An axial cross-sectional image of the lens showed an increase in SNR in the center of the lens and loss of symmetry compared with the normal lenses (Fig. 1B). The disruption on the posterior surface was thought to be the scar from the surgical trauma. The observed SNR change was attributed to an increase in both T1 and T2 (Fig. 2). Six weeks after cataract induction, biomicroscopic evaluation revealed 1+ anterior cortical and anterior subcapsular opacities and 2+ posterior subcapsular cataractous changes. The lens nucleus was clear. The proton density-weighted image (Fig. 1C) shows a regressed contrast pattern in the lens compared with that of the 2-week-old cataractous lens.

The images shown in Figure 2 are reconstructed pure T1 and T2 maps of the orbit (in color). The optimized color contrast for each map is shown. The T1 map of the normal lens showed a gradual decrease in T1 values from 1200 msec of the capsule to 300 msec in the center of the lens. The T1 relaxation in the center of the 2-week-old cataractous lens increased to 1000 msec with a T2 exceeding 30 msec. These

FIGURE 1. Proton density-weighted axial images (TE/TR, 8/1000 msec) of (A) a normal right orbit and a cataractous left orbit at (B) 2 weeks postsurgery and (C) 6 weeks postsurgery.
FIGURE 2. Computer reconstructed T1 and T2 maps of lenses: (A and B) normal lens, (C and D) 2 weeks postsurgery, and (E and F) 6 weeks postsurgery.
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Figure 3 contains the lens diffusion coefficient maps, computer generated, in the x (horizontal) and y (vertical) directions: (A and B) normal, (C and D) 2 weeks postsurgery, and (E and F) 6 weeks postsurgery.

Changes were presumed to be correlated with the increased water content, i.e., edema, in the lens. Figures 2E and 2F obtained from the 6-week-old cataract show decreased T1 and T2 in the center of a lens similar to normal lenses.

Figure 3 contains the lens diffusion coefficient maps of mobile protons sensitized to diffusion by gradients in the x (horizontal) and y (vertical) directions. In the normal lens, the lighter equatorial regions of Figure 3A show higher diffusion coefficients com-
A histopathologic preparation of the enucleated lens, 6 weeks postinjury, revealed loss of the normal lens architecture in the anterior cortical, posterior cortical, and posterior subcapsular regions (Fig. 4).

Figure 5 shows the regions of interest selected for T1, T2, and diffusion coefficient calculations. Table 1 summarizes the relaxation values obtained from similar regions of interest in a normal lens and both stages of a cataractous lens. The changes in T1 and T2 values were measured in the peripheral regions (ROI, 1–5 and 7) of the cataractous lens and were possibly the result of an alteration in the hydration pattern. The most notable changes in these values were, again, the increase in relaxation times in the nucleus (ROI, 6) of the 2-week old cataractous lens and the return to normal value at 6 weeks. Table 2 shows the values of diffusion coefficients in the same ROI. The diffusion coefficients in both the x and y directions in the lens nucleus were measurable only in the 2-week-old cataract. A consistent increase in the y-direction diffusion coefficient in the equatorial regions (ROI, 1 and 5) was observed in the cataractous lens compared with a normal one. The diffusion coefficients in the posterior pole (ROI, 7) were lower in both stages of the cataract, which could possibly be attributed to the change caused by the trauma.

DISCUSSION

In humans, perforating injuries of the lens typically lead to traumatic cataract. In some animals, including...
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TABLE 1. Selected Regional Spin-Lattice (T1) and Spin-Spin (T2) Relaxation Times of the Lens

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>T1 (msec)</th>
<th>T2 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 3)</td>
<td>Cataract 2 wk</td>
<td>Cataract 6 wk</td>
</tr>
<tr>
<td>1</td>
<td>1132 ± 25</td>
<td>956</td>
</tr>
<tr>
<td>2</td>
<td>803 ± 18</td>
<td>812</td>
</tr>
<tr>
<td>3</td>
<td>773 ± 16</td>
<td>770</td>
</tr>
<tr>
<td>4</td>
<td>819 ± 16</td>
<td>814</td>
</tr>
<tr>
<td>5</td>
<td>1142 ± 89</td>
<td>1086</td>
</tr>
<tr>
<td>6</td>
<td>447 ± 20</td>
<td>1107</td>
</tr>
<tr>
<td>7</td>
<td>892 ± 63</td>
<td>988</td>
</tr>
</tbody>
</table>

Normal values are mean ± SE.

rabbits, capsular healing occurs, resulting in a clear lens with the exception of a small localized scar. Some studies have reported that these species differences in the healing response may be the result of differences in the posttraumatic protein content of the aqueous fluid. Apparently, the posttraumatic protein content of the aqueous humor in humans is low relative to that of rabbit, thus lowering the chances of spontaneous wound healing. The ocular fibrin response often seen in rabbits after surgical manipulation or trauma is thought to facilitate closure of lens capsule defects.

The initial MRI changes seen after surgical trauma can be correlated with the swollen, overhydrated, opacified lens noted clinically. The MRI characteristics of this type of lenticular opacity are increases in both TI and T2 and altered diffusion patterns. Presumably, the MRI characteristics of the lens were markedly reversed because of closure of the traumatic lens defect and reestablishment of a more normal lens water content. MRI findings 6 weeks after the traumatic surgery are evidence of this healing process. Although slit-lamp micrographs were not obtained, the changes in MRI characteristics paralleled the lenticular changes noted clinically. The final MRI studies were correlated with lenticular histopathologic findings that revealed anterior and posterior cortical and posterior subcapsular changes. These studies and this illustrative case of a traumatic cataract suggest that high-resolution MRI can be used with a relatively high degree of sensitivity to follow the hydration status of the structural lattice and water movement in the lens over time in animal models. The advantages of MRI over the currently available techniques, especially fluorophotometric methods, of quantifying lens changes are that it is noninvasive and can be standardized for sequential studies by using set imaging parameters, such as T1, T2, and proton diffusion coefficients. The multiplanar capability of MRI allows precise localization of the lenticular lesions. In addition to the study of traumatic cataract, MRI presents us with a new and quantitative technique by which we may study the mechanisms in-

TABLE 2. Selected Regional Diffusion Coefficients in x- and y-Direction of the Lens

<table>
<thead>
<tr>
<th>ROI</th>
<th>Normal (n = 3)</th>
<th>Cataract</th>
<th>Cataract</th>
<th>Normal (n = 3)</th>
<th>Cataract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.13 ± 0.03</td>
<td>2.0</td>
<td>2.3</td>
<td>0.90 ± 0.06</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>1.35 ± 0.13</td>
<td>1.1</td>
<td>1.5</td>
<td>1.12 ± 0.09</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>1.99 ± 0.12</td>
<td>0.9</td>
<td>1.1</td>
<td>0.84 ± 0.08</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>1.47 ± 0.29</td>
<td>1.1</td>
<td>1.3</td>
<td>1.06 ± 0.11</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>2.25 ± 0.20</td>
<td>1.7</td>
<td>2.1</td>
<td>0.89 ± 0.06</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>2.3</td>
<td>—</td>
<td>—</td>
<td>1.3</td>
</tr>
<tr>
<td>7</td>
<td>0.95 ± 0.09</td>
<td>1.4</td>
<td>1.5</td>
<td>1.48 ± 0.18</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Normal values are mean ± SE.
olved in other lenticonal disorders. Extension to humans is technically feasible, in our judgment, but will require the design of new gradient coils, radiofrequency coils, and pulse sequences.

The avascular nature of the lens provides a unique tissue model for studying proton self-diffusion using high-resolution MRI. Intrinsic physical factors, such as regional fiber orientation, cellular density, intercellular barriers, and polar sutures, are likely to affect the restricted self-diffusion of water in the lens. In addition to the physical properties of the lens fiber lattice, regional cellular events, such as ionic currents, are factors that may be correlated with the measured diffusion anisotropy. Presumably, trauma-induced breakdown of the regional lattice architecture and disruption of the regional ionic current activities may be responsible for the observed changes in diffusion pattern. The diffusion measurements reported here were all made at the same TE; hence, the same time was allowed for diffusion to occur. At the TE used, the distance that water molecules diffuse is approximately 2–10 μm. Therefore, the characteristic size of the barriers that cause the observed anisotropic-restricted diffusion is on the same order. If a different TE were used, it is possible that the measured diffusion coefficients would also be different. The dependence of diffusion coefficients and anisotropy on TE should lend additional insight into the geometry of the structures that create restricted diffusion in the lens.

Measuring the opacity of the lens by biomicroscopic evaluation has been the standard method for staging cataracts in the past. Providing a quantitative alternative to the existing method, our in vivo method of measuring T1, T2, and the diffusion coefficient of the lens would also be different. The dependence of diffusion coefficients and anisotropy on TE should lend additional insight into the geometry of the structures that create restricted diffusion in the lens.

Key Words
magnetic resonance imaging, diffusion coefficients, spin–lattice relaxation, spin–spin relaxation, lens, traumatic cataract

References