Magnetic Resonance Imaging of the Rabbit Eye

Improved Anatomical Detail Using Magnetization Transfer Contrast

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Proton nuclear magnetic resonance (NMR) imaging previously has been used to examine structure and pathologies of the eye. The present study investigates the use of a saturation-transfer technique, which exploits water-macromolecular proton magnetic interactions, to enhance image contrast in the rabbit eye in vivo. Upon steady-state saturation of the macromolecular-proton magnetization, the water-proton signal intensity will decrease in proportion to the degree of water-macromolecular proton magnetic interaction. NMR images of the eye collected using saturation transfer are shown to have superior contrast compared to conventional NMR imaging techniques, in regard to numerous ocular structures, including the iris, ciliary bodies, muscle, lens, and cornea.

Nuclear magnetic resonance (NMR) imaging and spectroscopy are promising techniques for studying the physical structure and biochemistry of various regions of the eye. The different physiological characteristics of eye tissues that are important in function are also those that lead to differences in magnetic relaxation and contrast in NMR images. The function of eye tissues, particularly the lens, are critically dependent on the structure and hydration of the macromolecular matrix, the same characteristics that enhance water-proton spin relaxation. By assessing magnetic relaxation with imaging and spectroscopy under a variety of conditions, a better understanding of eye physiology may emerge. Moreover, these studies may establish magnetic resonance as a new tool in assessing various diseases and pathological development in the eye.

The enhancement of water-proton magnetic relaxation in the presence of macromolecules has been studied extensively. Mechanisms that contribute to this enhanced relaxation include long-range hydrodynamic effects, exchange of water molecules or protons between bulk water and macromolecular hydration environments, and through-space dipolar coupling between water and macromolecular protons at the macromolecular surface (cross relaxation).

The imaging technique employed in the present work exploits the dipolar coupling, or cross relaxation, between water and macromolecular protons to enhance image contrast. Cross relaxation is an efficient relaxation mechanism for water protons. However, it is present only with selected macromolecules. (Preliminary evidence from our lab suggests that the presence of a hydroxyl group on the macromolecular surface is a necessary condition for cross relaxation to occur.) Contributions to water-proton relaxation by cross relaxation with macromolecular protons can be determined using saturation-transfer techniques. This method involves the selective saturation of the macromolecular proton magnetization with low-power radio-frequency irradiation, which results in a decrease in the water-proton equilibrium magnetization through water-macromolecular proton magnetization transfer. The water-proton spin-lattice relaxation in the presence of macromolecular proton saturation ($T_{1\text{sat}}$) will be shorter than in the absence of saturation ($T_{1\text{obs}}$). The degree of relaxation enhancement and the magnitude and rate of magnetization transfer are affected by the dynamics of the water/macromolecular complex, which are directly related to structure, dynamics, and surface chemistry.

These saturation-transfer techniques can be used in an imaging modality where alterations in image contrast upon macromolecular proton saturation relate to the degree of water-macromolecular proton magnetic coupling, termed magnetization transfer contrast or MTC. Because only certain tissues will demonstrate this effect, magnetization transfer imaging is a selective probe of tissue structure and dynamics. $T_{1\text{sat}}$ images can be acquired and used to calculate magnetization transfer rate maps, which may provide further qualitative and quantitative assessment of tissue characteristics.

The present work investigates the application of MT imaging techniques to the evaluation of the eye in...
vivo. This approach provides a unique noninvasive view of the morphology and pathology of the eye, based on the magnitude and dynamics of water-macromolecular interactions.

**Materials and Methods**

**Animal Preparation**

All experiments were performed on New Zealand White rabbits of both sexes weighing between 1.5 and 3 kg. Animals were initially sedated with an intramuscular injection of acepromazine (1.5–3 mg)/ketamine (150–300 mg), then intubated and ventilated. The rabbits were anesthetized throughout the imaging procedure using 1.0–1.5% halothane. Pavulon (0.5 mg) was administered every half hour to reduce muscle movement around the eye. The use and treatment of the animals complied with the ARVO Resolution on the Use of Animals in Research.

**Magnetic Resonance Techniques**

NMR studies were conducted on a General Electric Omega spectrometer (GE NMR Instruments, Fremont, CA) interfaced to a horizontal 4.7 Tesla Oxford magnet (Oxford Instruments, Oxford, England) with a 26 cm clear bore. The rabbit was placed in a special cradle to support the animal in the magnet. A single turn 2.5-cm transmit/receive surface coil was positioned over the closed eye and tuned and matched to 200.123 MHz and 50 ohms respectively. The cradle was placed in the magnet and the eye centered within the homogeneous field.

The saturation transfer experiment was carried out by applying low-level rf-irradiation 10 kHz off-resonance from the free-water proton signal to selectively saturate the broad resonance from the macromolecular protons. Under conditions of complete (and instantaneous) saturation, the equilibrium magnetization for the coupled water protons is given by

\[
\frac{M_s - M_0}{M_0} = - \frac{T_{\text{sat}}}{T_{\text{MT}}}
\]

where \(M_s\) and \(M_0\) are the water-proton equilibrium magnetizations measured in the presence and absence of saturation of the macromolecular proton magnetization. \(T_{\text{sat}}\) is the water-proton spin-lattice relaxation time measured in the presence of saturation. \(1/T_{\text{MT}}\) is the pseudo-first-order rate constant for magnetization transfer into the saturated pool. The magnetization transfer effect is rapidly transferred to the bulk-water phase through exchange of water molecules at the surface and diffusion. \(1/T_{\text{MT}}\) will include the rate for magnetization transfer at the water-macromolecular interface and the rate for equilibration into the bulk pool.

Magnetization transfer is extended to an imaging modality in standard spin-echo and gradient-recalled-echo sequences, with saturation applied during the predelay and echo-delay periods. Using the surface coil for transmit and receive, gradient-recalled-echo (GE) images were acquired with and without off-resonance saturation, as illustrated in Figure 1. GE images were slightly \(T_1\)-weighted with a spin-echo delay (TE) of 13 msec and a recycle time (TR) of 1 sec. The saturating field was applied through the decoupler channel of the spectrometer +10 kHz from the water proton resonance. A saturating field of \(1 \times 10^{-5} \text{T}\) at +10 kHz from the water resonance provides near complete saturation of the macromolecular proton resonance with minimal bleedover saturation of the narrow bulk-water proton resonance.\(^{16,17}\) The saturating field amplitude was adjusted so that it was approximately \(1 \times 10^{-5} \text{T}\) at the level of the lens.

Standard spin-echo \(T_1\) and \(T_2\)-weighted images were also collected in this study. A Helmholtz coil positioned around the rabbit’s head was used for homogenous rf transmit pulses and the surface coil was positioned on the eye for signal reception. The saturating field was calibrated to \(\sim 1 \times 10^{-5} \text{T}\) by determining the 180° pulse width at the bulk water resonance.

A magnetization-transfer rate constant image was generated based on equation 1. The \(T_{\text{sat}}\) image data were acquired using one-shot \(T_1\)-imaging,\(^{20}\) employing the surface coil for transmit and receive. This sequence employs an inversion pulse followed by a series of small tip-angle pulses that sample the relaxation curve as the magnetization approaches equilibrium. An adiabatic pulse was used for homogeneous inversion across the volume sampled by the surface coil.\(^{21}\) The sampling interval was 0.8 sec, eight images were acquired, and the sampling-pulse tip angle was adjusted to be 20° or less across the volume sampled by the surface coil. Time constants were calculated pixel by pixel assuming a single exponential decay using IDL software, (Research Systems, Inc.,

![Fig. 1. Schematic representation of the saturation transfer experiment incorporated into a standard gradient-recalled-echo imaging sequence.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933155/ on 06/06/2017)
Under the acquisition conditions stated above, for $T_{1\text{sat}}$ values of 2 sec or less, the error in equating the calculated time constants from the one-shot imaging sequence to $T_{1\text{sat}}$ is less than 15%. The images acquired at the last sampling interval in the $T_1$ and $T_{1\text{sat}}$ data sets were used as the $M_s$ and $M_o$ images in equation 1. The rate constant image is effectively noise filtered by setting all values above 20 and below 0 to 0. The total acquisition time for the $T_1$ images was approximately 1 hr, which is less than that required for inversion recovery or saturation recovery methods.

Results and Discussion

Standard GE images of the rabbit eye without and with off-resonance saturation are presented in Figure 2. Gradient-recalled-echo images of the in vivo rabbit eye. The dark artifacts seen in the upper right surface of the eye are a result of susceptibility artifacts at a point where the eyelid was partly open and the surface of the eye was dry. A linear scale has been applied to the images to enhance the intensity in regions far from the plane of the surface coil. (A) Image acquired without saturation of the broad lipid proton resonance. Acquisition parameters were TR/TE 1 sec/13 msec, 256 × 256 image resolution, 40 mm full field of view, and 8 acquisitions per phase encode step. (B) Image acquired with the same parameters as (A) with a 1 sec $1 \times 10^{-5}$ T field applied +10 KHz from the bulk water proton resonance during TR.

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Fig. 3. The difference image between Figures 2A and 2B ($M_o - M_s$). Labeled anatomy: A, lens cortex; B, lens nucleus; C, iris (striated muscle, collagen, epithelium); D, posterior chamber; E, ciliary processes (collagen, vascular tissue, epithelium); F, cornea (collagen); G, anterior chamber; H, sclera (collagen); I, choroid (connective and vascular tissue); J, neural retina (neural cells, membranes); K, vitreous body (water, collagen).

Fig. 4. Standard spin-echo images of the in vivo rabbit eye. (A) Proton density spin-echo image (TR/TE 1 sec/13 msec), 256 × 512 image resolution (zero filled to 512 × 512), 8 acquisitions per phase encode step. (B) $T_2$-weighted image (TR/TE 2 sec/50 msec), 256 × 512 image resolution (zero filled to 512 × 512), 8 acquisitions per phase encode step.
2. Water proton signal intensity is decreased in areas where there is significant water-macromolecular interaction. These areas are most clearly seen in a difference image (2a-2b) shown in Figure 3. Several structures show a high degree of coupling between the water and macromolecular protons, including the lens, iris, ciliary bodies, skeletal muscle, and choroid.

The contrast in the magnetization transfer and difference images is superior to standard T\textsubscript{1}- and T\textsubscript{2}-weighted images. The heavily T\textsubscript{1}-weighted image (not shown) shows a loss in signal intensity with no increase in contrast compared to the proton density image (Figures 2a and 4a). The magnetization transfer image (Figure 2b) shows higher contrast and greater detail in the iris and ciliary bodies compared to the T\textsubscript{2}-weighted (Figure 4b) and proton density images. The greatest contrast is seen in the difference image (Figure 3), where only structures with high water-macromolecular interactions are visualized. In this difference image there is complete cancellation of signal in the vitreous and anterior and posterior chambers, more of the lens nucleus is visualized, contrast around the iris and ciliary bodies is further enhanced, and the transition from sclera to cornea is seen. The magnetization transfer image has a higher signal-to-noise ratio and is acquired in a shorter time compared to the T\textsubscript{2}-weighted image. This is a result of shorter TE and TR times, respectively.

A magnetization-transfer rate map and corresponding images, acquired as for Figure 2, are presented in Figure 5. Of note is the absence of signal in the rate image in the lens nucleus, a region of high intensity in the M\textsubscript{0}-M\textsubscript{0} difference image (Fig. 5c). In the limit of maximum coupling between the water and macromolecular proton systems, the calculated rate \( \kappa \) approaches \( 1/T\text{lsat} \) and M\textsubscript{0} approaches zero. Under these conditions, the rate cannot be determined because, as a result of M\textsubscript{0} approaching zero, T\text{lsat} cannot be measured. The value of \( \kappa \) in the lens cortex ranges from 0.5 to 1.3 sec\textsuperscript{-1}.

The high resolution images presented in Figures 2 and 3 were acquired in approximately 34 min. This is a long time in today’s imaging standards. However, this may be justified where microscopic abnormalities may be present. Nearly the same detail is obtained in the images presented in Figure 5, which have half the resolution and were acquired in approximately 8 min.

It has been shown in lipid bilayer suspensions that the chemistry and the rigidity of the macromolecules are critical for the effectiveness of the magnetization-transfer coupling.\textsuperscript{22} These studies and others have suggested that hydroxyl groups and other exchange-
able groups on the macromolecule are key sites in this interaction.\textsuperscript{11,23,24} This may explain why eye structures containing significant fractions of collagen demonstrate high magnetization transfer coupling; they are rigid and have high levels of external hydroxyl groups.

Regarding the lens, these preliminary results suggest that magnetization transfer effects may provide information on the extent of protein hydration and chemistry with respect to age and pathology. As seen in the difference image presented in Figures 3 and 5c, the lens possesses a strong interaction between water and macromolecular protons. The decreasing intensity in the nucleus of the lens reflects the lower lens hydration of the nucleus rather than relaxation effects. The extracellular space in the lens has been estimated to be about 1%. The lens fiber cells are high in protein (>35%) and their plasma membrane is high in cholesterol and sphingomyelin,\textsuperscript{25,26} which have been demonstrated to be extremely efficient in coupling to the bulk water.\textsuperscript{17} Thus, the physical chemistry of the lens is extremely conducive for the coupling of macromolecules and water protons, as observed in this study. The combination of the qualitative \( M_2 - M_1 \) difference image and the quantitative magnetization transfer rate image fuels anticipation that various disease states of the lens involving lens hydration changes or osmotic cataract formation can be detected.

In summary, magnetization transfer imaging provides a superior method for generating contrast in MRI images of the eye in vivo. These studies were conducted at 4.7T, but other reports from this laboratory suggest these effects should be similar at lower, more clinically useful fields (1 to 2T). This type of contrast may prove useful not only in following the morphology of the eye but in offering insights into the physical chemistry of the lens and other structures within the eye during various disease states.

**Key words:** surface coil, spin-lattice relaxation, macromolecules, collagen, lens, spin-echo, gradient-recalled echo

**References**