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Visualization of water movement in the living rabbit eye

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Abstract. Water enriched with the stable isotope ^{17}O ($H_2^{17}O$) shortens the transverse relaxation time (T2) of protons in water and can therefore be used as the contrast agent for proton magnetic resonance (MR) imaging. This agent can be given topically or intravenously to demonstrate water movement in the eye. Topical $H_2^{17}O$ (0.05–0.1 ml/eye, 10% enrichment) entered the anterior chamber within 5 min and dissipated from the chamber in a single-exponential fashion (flow-rate constant $k = 0.1\ min^{-1}$), principally due to an exchange with the iridic circulation. No $H_2^{17}O$ was detected in the vitreous. Intravenous administration of $H_2^{17}O$ (1 ml/kg, 10% enrichment) resulted in rapid entry (<20 min) of the agent into the aqueous chamber. Again, no $H_2^{17}O$ was detected in the vitreous. The lens region, on the other hand, showed an increase in image intensity with time that reached a plateau after 40 min. Although these findings are preliminary, acetazolamide (20 mg/kg injected intravenously) appeared to affect iridic circulation, possibly through vasoconstriction. Potential application of this $H_2^{17}O$-enhanced MR imaging technique is discussed.

Introduction

The aqueous flow pattern in the eye has been demonstrated by McLaren and Brubaker [14] using two-dimensional fluorophotometry. More recently, proton magnetic resonance (MR) imaging using gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) as the contrast agent to follow the aqueous flow of the eye has been achieved [1, 3]. However, both fluorescein and Gd-DTPA differ from water not only in chemical properties but also, on a more basic level, in diffusivity: both comprise much larger molecules than water, with respective molecular weights of 510 and 550 kDa, and neither can diffuse across biological membranes. The distribution pattern of these agents must therefore be different from that of water. In fact, this difference was pointed out by Kinsey et al. as early as in 1942 [10].

We have found that water enriched with the stable isotope ^{17}O ($H_2^{17}O$) can be used as the contrast agent for proton MR imaging of water flow in the eye [1], as it shortens the transverse relaxation time (T2) of protons in water [8, 9]. Since free, unbound water in tissue displays the longest T2 (on the order of 3 s), any decrease attributable to $H_2^{17}O$ results in good contrast. In addition, the eye is ideally suited for T2-weighted imaging: when acquired with long echo times (TEs), tissue signals are diminished, whereas the aqueous and vitreous humors remain bright. The appearance of $H_2^{17}O$ is easily detected on its entry into the pool of free, nonenriched water [nonenriched water refers to water that contains only naturally abundant ^{17}O (0.037%) and has not been artificially enriched], i.e., that in the aqueous or vitreous.

Furthermore, the proton signal intensity in the pool decreases in direct proportion to the increasing concentration of $H_2^{17}O$. High-resolution proton MR imaging can therefore be used to detect and quantify the flow of water in the living eye. We tested the feasibility of using this technique to trace the movement of water in the living eye, and our results are discussed in this report.

Materials and methods

New Zealand White rabbits (2 kg body weight) were purchased from a local supplier. The care of these animals conformed to the institutional guidelines at Massachusetts Eye and Ear Infirmary. Anesthesia was achieved by the intramuscular injection of a mixture of ketamine (40 mg/kg) and xylazine (10 mg/kg). For experiments requiring rabbits that showed no cardiac output, the animals were killed with an overdose of pentobarbital.

Normal saline prepared with $H_2^{17}O$ (10% enrichment, obtained from Cambridge Isotope Laboratories, Woburn, Mass.) was applied topically (0.05–0.1 ml/eye) or intravenously through the ear vein (1 ml/kg) in anesthetized animals that had been positioned in a 1.5-T clinical imager (General Electric, Milwaukee, Wis.). Sequential spin-echo proton imaging was then conducted and the signals were received by means of a 3-inch surface coil. The MR

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imaging parameters were: TR (repetition time)/TE (echo time)= 1,000/360 ms for the topical study and TR/TE=1,500/800 ms for the intravenous study, both of which used a slice thickness of 4 mm. The inplane resolution was 0.5×0.5 mm. (Multi-echo images were actually obtained, with the TEs indicated above providing the best contrast.)

When used, acetazolamide (Lederle Laboratories, Pearl River, N.Y.) was injected through the ear vein at a dose of 20 mg/kg. In the intravenous $H_2^{17}O$ study, some rabbits ($n=2$) were used in two sessions; the eyes were examined in one session and the rabbits were then kept for 2 weeks to allow the excretion of $H_2^{17}O$ from the body system. Acetazolamide was given at the beginning of the second session at 2 h before imaging.

Measurement of image intensity was performed using the standard region-of-interest software. To minimize volume-averaging artifacts, a 0.01-cm^2 circular cursor was used to define regions in the anterior chamber, in the posterior cortex of the lens, and in the core of the vitreous. In some cases, the peripheral vitreous was also measured using the same program. As determined in a preliminary study, the MR image intensity was a linear function of the concentration of $H_2^{17}O$ within the range of 0.1%–5%, which agreed with the results of Hopkins and Barr [8].

Results

Topical route

$H_2^{17}O$ entered the anterior chamber rapidly (<5 min). The MR images showed a decrease in signal intensity in the anterior chamber (Fig. 1A). This low aqueous signal intensity stayed constant for about 10 min, followed by a recovery period (Fig. 1B). The constant period was shortened or totally eliminated when the eyes were blotted dry after the initial $H_2^{17}O$ loading. The "recovery" usually reached a plateau at 20 min after its onset and could be characterized by a single-exponential decay function [11]: Signal intensity $= A + B\,e^{-kt}$, where A and B are constants, t is time, and k is the flow-rate constant. We found that $k = 0.1 \pm 0.02\ ml^{-1}\,min^{-1}\,ml^{-1}$ ($n=3$). No signal change was found in other parts of the eye such as the crystalline lens and the vitreous. In dead rabbits, the entry of $H_2^{17}O$ into the anterior chamber could be seen but no recovery was detected. The recovery was therefore circulatory in origin. This flow pattern was observed in all rabbits tested ($n=7$).

The rate of disappearance of $H_2^{17}O$ from the anterior chamber after intracameral injection was identical to that found after topical application. The former procedure was carried out by injecting 10% $H_2^{17}O$ at 0.1 ml/eye directly into the anterior chamber ($n=3$) following the removal of an equivalent amount of aqueous humor (volume of the anterior chamber ≈ 0.2 ml). In dead rabbits, intracameraly injected $H_2^{17}O$ was seen to invade the anterior vitreous from the posterior aqueous chamber.

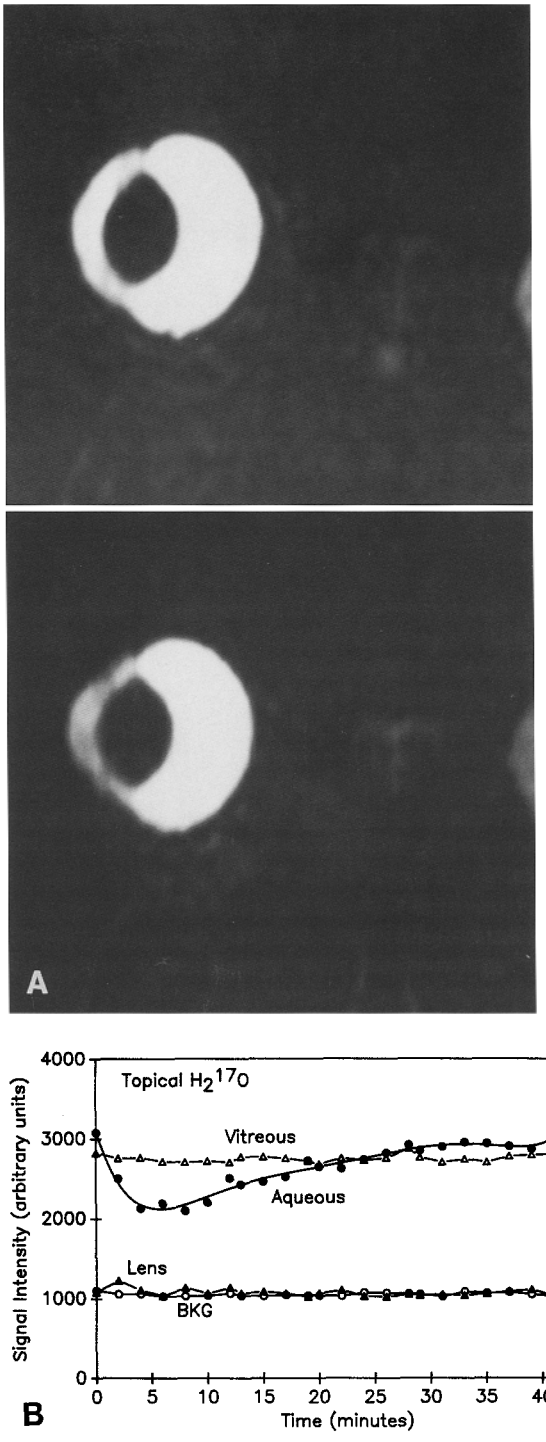
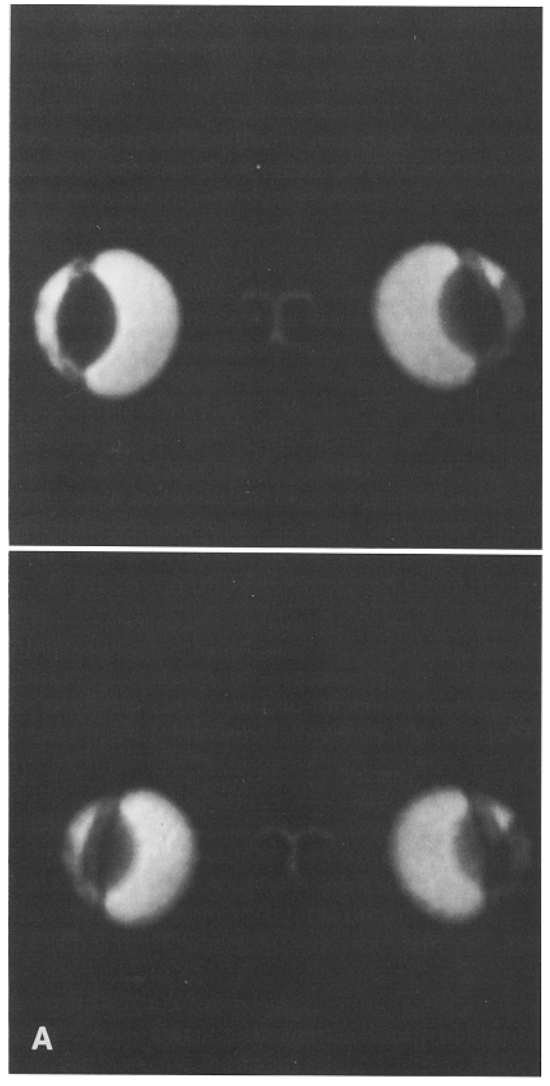


Fig. 1A, B. Topical application of 10% $H_2^{17}O$ at 0.05 ml/eye. **A** Eye as visualized before (*top*) and after (*bottom*) application. Note the loss of signal intensity in the anterior chamber after $H_2^{17}O$ administration. **B** Plot of signal intensity in the aqueous, lens and vitreous vs time. *BKG*, Background of the images (signals=0)

Intravenous injection

As with the topical method, after the intravenous injection of $H_2^{17}O$, the anterior chamber showed a decrease in signal intensity (Fig. 2A). This process was completed within 20 min (Fig. 2B), after which the signals remained constant. The time course was similar to that of the topical study; however, because of the long image-acquisition time (6.5 min/image when TR/TE=1,500/800 ms), the kinetics could not be established accurately – this is to be remedied with fast imaging techniques



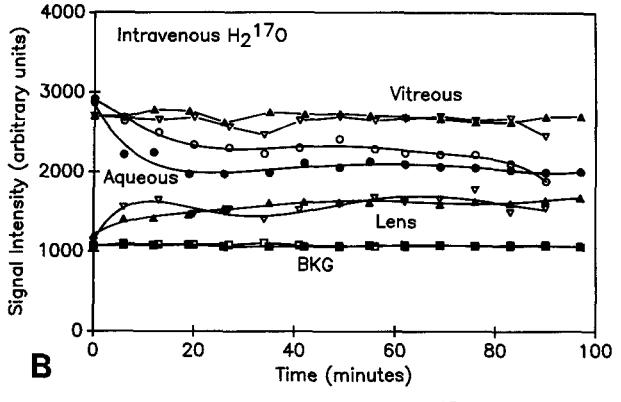


Fig. 2A, B. Intravenous injection of 10% $H_2^{17}O$ at 1 ml/kg. **A** Eye as visualized before (*top*) and after (*bottom*) injection. Note that the iris-ciliary body appearing as a dark band (*top*) is highlighted (*bottom*) due to the increased lens signal intensity in the background. The lower anterior part of the eye on the *right* is obscured by the nictating membrane. **B** The change in image intensity with time. *Filled symbols*, Before acetazolamide treatment; *open symbols*, after treatment. Data were obtained from the same rabbit in two imaging sessions carried out 2 weeks apart. *BKG*, 0 signals

currently capable of image acquisition on the order of ≈ 30 ms/image [9]. The vitreous (the core as well as the periphery) showed no change in signal intensity; in contrast, the lens region displayed an increase that reached a plateau after 40 min (Fig. 2B). This flow pattern (Fig. 2B) was also reproducible ($n=4$).

After a 2-week recovery period, two rabbits from the control series were subsequently used in the acetazolamide study. The results showed that the rate of aqueous uptake of $H_2^{17}O$ was retarded 2-fold, with the $H_2^{17}O$ level measured being 30% lower (Fig. 2B, indicated by a higher proton signal intensity) than that observed 2 weeks previously.

Discussion

The appearance of $H_2^{17}O$ in the anterior chamber is not unexpected, as water can diffuse across biological membranes. Its removal from and entry into the anterior chamber principally reflect an exchange of water between the aqueous humor and the iridic tissue and circulation and, to a lesser extent, the flow through the aqueous outflow channels. Indeed, the flow-rate constant for $H_2^{17}O$ is 0.1 min^{-1}, which is identical to that previously obtained using D_2O as the tracer [4] and about 10 times faster than the "aqueous" flow as determined by the Gd-DTPA method [3] or fluorophotometry [15].

Using the $H_2^{17}O$-enhanced MR imaging technique, it may be possible to evaluate disturbances of anterior segment circulation and drug-induced vascular changes in the iris (and ciliary body). Although these findings are preliminary, acetazolamide appears to exert some effect on the influx of intravenous $H_2^{17}O$ into the aqueous chamber (Fig. 2B). This carbonic anhydrase inhibitor is also supposedly a vasoconstrictor [12, 13], which can then impede water exchange between the iridic circulation and the aqueous humor. (Note: The observed effect, if substantiated, apparently was not due to the base content associated with acetazolamide preparations. The vasoconstrictive effect of the base reportedly lasts up to 1 h [15], whereas in the present study, MR imaging was performed at 2 h after acetazolamide injection.)

We recorded two interesting findings after $H_2^{17}O$ had been injected intravenously: (1) the vitreous showed no signal change and (2) the lens region displayed an increase in image intensity. It is possible that the vitreous actually exchanges its water with the uvea and that this process is demonstrable only if sufficient $H_2^{17}O$ has been loaded and/or if the images are acquired within seconds. The dose of 1 ml/kg 10% $H_2^{17}O$ used in this study probably did not enable visualization of the water exchange. Even had it done so, the rapidity of uveal blood flow [7] would not have enabled the entry/exit of $H_2^{17}O$ into/out of the peripheral vitreous to be recorded, as the imaging time was 6.5 min/image. Nevertheless, in comparison with the aqueous humor, the vitreous/uveal water exchange is clearly a very different process at the $H_2^{17}O$ dose used. Further studies are currently in progress.

We found that intracamerally injected $H_2^{17}O$ diffused into the vitreous only in dead rabbits; this implies either the presence of a diffusion barrier between the posterior chamber and the anterior vitreous under normal conditions or a rapid removal of $H_2^{17}O$ by uveal circulation.

The increase in signal intensity in the lens region cannot be explained by a change in the transverse relaxation time (T2) of water protons in the lens, as the overall T2 of the lens is extremely short, being on the order of 20–30 ms [2]. We have recently found that this increase was due to the displacement of $H_2^{16}O$ by $H_2^{17}O$ from the lens equator into the posterior chamber [5] and that the increasing intensity originated from the $H_2^{16}O$ in the latter. The absence of a bulk flow of water from the lens into the vitreous, however, is totally unexpected. It is well known that a pump-leak mechanism for K^+ ions exists in the lens in the direction of aqueous→lens→vitreous [6]. Our data indicate that there is extremely slow communication, if any, between the lens and the vitreous. We noticed that topical application did not induce any signal change in the lens region (Fig. 1), as much less $H_2^{17}O$ (considerably less than the topical dose of 0.05–0.1 ml/eye) would have entered the anterior chamber.

The results reported herein demonstrate the feasibility of $H_2^{17}O$-enhanced magnetic resonance (MR) imaging and the possibility of quantifying the water flow. Further quantitation of the flow process must be established for the clinical application of this technique to be practical. It is important to note that MR imaging using $H_2^{17}O$ can currently be applied to humans: (1) the current imaging equipment (commonly at 1.5 T) is adequate, although fast imaging can further improve the accuracy of the kinetic data while simultaneously reducing motion artifacts; and (2) $H_2^{17}O$ has no known toxicity [8] and can be applied expediently in the form of eyedrops or injectable agents or, possibly, through oral intake. However, because of the relatively high cost and scarcity of $H_2^{17}O$, its topical application promises to be the most clinically relevant approach. Information on water movement in the eye can be obtained in one imaging session.

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