7-1-1996

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Contrast-Enhanced Magnetic Resonance Imaging Confirmation of an Anterior Protein Pathway in Normal Rabbit Eyes

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Purpose. Contrast-enhanced proton magnetic resonance imaging (^{1}H MRI) has been used as a quantitative, noninvasive method to corroborate a pathway for the diffusion of plasma-derived protein into the aqueous humor in the normal rabbit eye.

Methods. T1-weighted magnetic resonance images were produced over 1- to 3-hour periods after the intravenous injection of gadolinium diethylenetriamine-pentaacetic acid.

Results. Analysis of the images yielded the time dependence of signal enhancements within the areas of interest. The ciliary body showed an immediate sharp increase, followed by a gradual decrease in signal enhancement with time. Although a gradual increase in signal enhancement was found in the anterior chamber, no significant change occurred in the posterior chamber. A similar MRI experiment with an owl monkey produced parallel, though smaller, signal enhancements in the ciliary body and anterior chamber. Again, however, no significant change was found in the posterior chamber.

Conclusions. These results support and extend those of recent fluorophotometric, tracer-localization, and modeling studies demonstrating that in the normal rabbit and monkey eye, plasma-derived proteins bypass the posterior chamber, entering the anterior chamber directly via the iris root. Invest Ophthalmol Vis Sci. 1996;37:1602–1607.

Recent fluorophotometric and tracer-localization studies in rabbit[1] and monkey[2] eyes indicate that plasma-derived protein in the aqueous humor of the normal eye enters the anterior chamber directly, diffusing from the ciliary body stroma through the iris root. Extensive computational modeling of protein diffusional mechanics, which was an integral part of these studies, indicated that most of the protein in anterior chamber aqueous humor is plasma-derived protein entering through the iris root and bypassing the posterior chamber. McLaren et al[3] have since confirmed that the addition of a third compartment (corresponding to the iris stroma) to earlier two-compartment models of anterior segment fluorescein kinetics significantly improves the correspondence of experimental and modeled fluorophotometric data in rabbits and humans. Despite this concordance, these data inherently are limited by the fact that reliable measurements from the posterior chamber cannot be obtained in vivo using fluorophotometric methods.

More recently, Mestriner and Haddad[4] have challenged the existence of an anterior diffusional pathway for proteins, maintaining that plasma-derived macromolecules traverse the blood–aqueous barrier of the ciliary epithelium in the normal rabbit eye and enter the posterior chamber.[4] Although concerns have been raised regarding the validity of the methods used in this study,[5] the issue of whether plasma-derived proteins enter or bypass the posterior chamber in vivo has not been resolved.

Verification and characterization of the presence of a major diffusional pathway across the iris root are

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Supported by Dreyfus Foundation grants and by National Institutes of Health grants AREA Program 1-R15-EY08548 and 1-R15-EY10615 (NHK, BAL, CS), RO1-EY04567 (TFF, SPB), and RO1-EY04914 (SPB).

Submitted for publication November 9, 1995; revised February 13, 1996; accepted March 21, 1996.

Proprietary interest category: N.

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important because this pathway probably has a significant role in the physiology of the trabecular meshwork and the diffusional kinetics of immunomodulators synthesized in the ciliary body stroma. Our goal in the current study was to address this issue noninvasively and in vivo through the use of high-resolution magnetic resonance imaging (MRI) after intravascular injection of the contrast agent gadolinium diethylenetriamine-pentaacetic acid (Gd-DTPA). Contrast in magnetic resonance images results from the tissue variations of the inherent relaxation times (T1 and T2) of the protons in water molecules. Contrast agents are exogenous compounds that may be administered to alter these protons' inherent relaxation rates ($T1^{-1}$ and $T2^{-1}$). Dynamic ocular processes that have been studied in vivo using contrast agents include aqueous humor flow[6-8] and permeability of the blood–retinal barrier.[9-14]

The power of MRI is that it allows the three-dimensional localization of dynamic ocular processes, even within small structures, such as the posterior chamber, that are unobservable by other in vivo methodologies, such as fluorophotometry. Contrast-enhanced MRI data obtained directly from the ciliary body and the anterior and posterior chambers of a normal rabbit eye were used in this study to validate the proposed diffusional pathway for plasma-derived proteins.[15]

METHODS

Animal Preparation

All animals used in this study were treated in strict accordance with institutional guidelines and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Four pigmented rabbits, each weighing between 1.5 and 3 kg, and one owl monkey (*Aotus trivirgatus*), weighing 1 kg, were used.

Magnetic resonance imaging studies are not associated with either pain or discomfort; however, the animals were anesthetized before their positioning inside the magnet to minimize extraneous motion during the study. Anesthesia for the rabbits was accomplished by subcutaneous administration of ketamine HCl (35 mg/kg body mass) and xylazine (5 mg/kg body mass). Anesthesia was maintained by intramuscular injection of ketamine HCl. The owl monkey was anesthetized with ketamine HCl (10 mg/kg body mass, intramuscular). A femoral vein was catheterized, and supplemental anesthetic, pentobarbital sodium (5 mg/kg body mass, intravenous) was administered as needed.

After sedation and anesthesia, the animal was placed in a plastic "bed" especially designed to position the eye and the MRI surface coil within the superconducting magnet. The contrast agent Gd-DTPA (0.5 mmol/ml, 0.4 ml/kg; Magnevist; Berlex Labs, Wayne, NJ) was administered by bolus injection with equal amounts of saline into the rabbit's ear vein through a catheter. The contrast agent for the monkey was delivered through the femoral vein catheter. After the completion of the experiments, the animals were allowed to awaken and were monitored to ensure a safe recovery.

Magnetic Resonance Imaging Procedure

All ^{1}H MRI experiments were performed on an imaging system at the Magnetic Resonance Imaging Research Division of the Radiology Department of the Brigham and Women's Hospital. The Division maintains at 4.7 T 30-cm bore animal imaging system, equipped with an Oxford (Oxford, UK) magnet and Oxford gradient coils. The magnet is interfaced to a GE CSI System (GE NMR Instruments, Fremont, CA) that includes a Sun 3 computer (Sun Microsystems, Mountain View, CA), CSI software, and the appropriate radiofrequency hardware for MRI. An inductively coupled surface coil (diameter 3.7 cm) was designed and built for use in our research. This coil was optimized to produce excellent images of the eye.

^{1}H MRI experiments were designed to produce very high-resolution (0.3×0.3 mm^2 in-plane; 2 mm slice thickness) T1-weighted images of rabbit eyes. Multislice spin echo pulse sequences, with echo and repetition times chosen to optimize image contrast, were used. T1-weighted images were acquired with echo times (TE) of 19 msec and repetition times (TR) of 400 msec, two data averages, 256 phase encode gradients, and a field of view of 75 mm. Image data acquisition times were between 4 and 5 minutes.

Before acquisition of image data, the magnetic field was shimmed on a phantom consisting of a set of solutions of contrast agent prepared in basic salt solution. After placement of the animal within the magnet, a low-resolution scout image was obtained, followed by final adjustments to verify animal positioning and slice selection. A high-resolution T1-weighted image was acquired of the rabbit or monkey eye before administration of the contrast agent. This image provides baseline values for quantitative interpretation of image signal intensities of various structures within the eye. Then, after administration of the contrast agent, image data were acquired over time periods chosen for best monitoring of blood–aqueous barrier permeability. Preliminary data showed that a complete follow-up of the diffusion of the contrast agent could be accomplished within a 120-minute period.

Image analysis was performed on a Macintosh IIci computer using the program *Image* (W. Rasband, National Institutes of Health, v. 1.57). The average signal intensity was measured in defined regions of interest

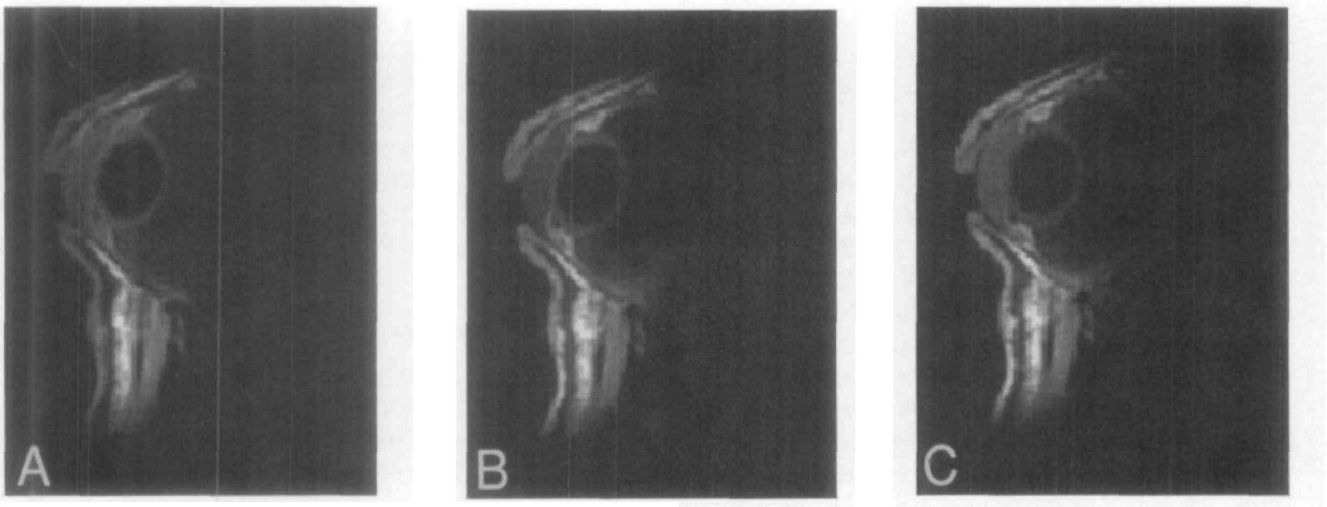

FIGURE 1. ^{1}H magnetic resonance images of a pigmented rabbit eye (**A**) before, (**B**) 3 minutes after, and (**C**) 39 minutes after intravenous administration of Gd-DTPA.

(ROI) within each of the areas under study—the ciliary processes and the anterior and posterior chambers. The average number of pixels analyzed within the ROI differed from structure to structure: anterior chamber, 120; ciliary processes, 20; and posterior chamber, 5. Pixels were selected in each ROI that lay completely within the boundaries of the structure to avoid signal averaging. The images were of sufficient clarity and resolution to allow this to be done easily on anatomic grounds alone, with care taken to avoid the enhanced signal intensity outlining the tissues that border this compartment (e.g., lens). All image intensities were normalized before analysis, and intensity values given are means ± SD. Graphic analysis of the data was performed using Excel (v. 4.0; Microsoft, Redmond, WA) and displayed as plots of percent signal enhancement over time for each ROI.

RESULTS

Figure 1 represents a typical series of Gd-DTPA-enhanced images of an eye of one of the pigmented rabbits used in this study. Regions of interest chosen from the image are the ciliary process and the anterior and posterior chambers.

The enhancement, E, of the MRI signal for any ROI is calculated by measuring the signal intensity at time t, S(t), and comparing it with the signal intensity at a control time, S_0: $E = (S(t) - S_0)/S_0$. Figure 2 shows the mean percent signal enhancement, E, as a function of time for the ciliary body and the anterior and posterior chambers for the four pigmented rabbits. A sharp increase in signal enhancement within the ciliary body can be seen immediately (t = 0 to 10 minutes) after intravenous Gd-DTPA administration. After 10 to 20 minutes, the signal enhancement reaches a maximum and is followed by a gradual decrease. Signal enhancement in the anterior chamber increases slowly throughout the course of the experiment, leveling off after approximately 60 to 80 minutes. In the posterior chamber, however, no significant signal enhancement is found. Figures 3 and 4 show parallel results from the owl monkey.

DISCUSSION

A diffusional pathway for plasma-derived proteins from the ciliary body stroma to the anterior chamber

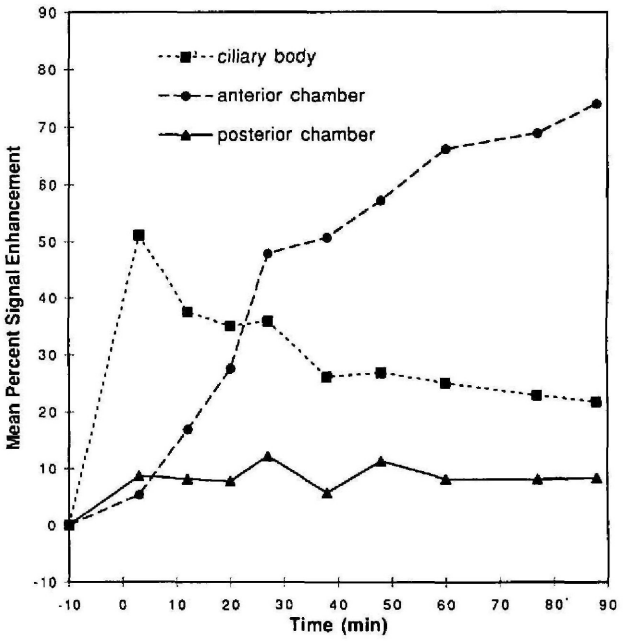

FIGURE 2. Mean percent signal enhancement, E, as a function of time after the administration of Gd-DTPA (at $t = 0$) for the ciliary body and the anterior and posterior chambers of four pigmented rabbit eyes.

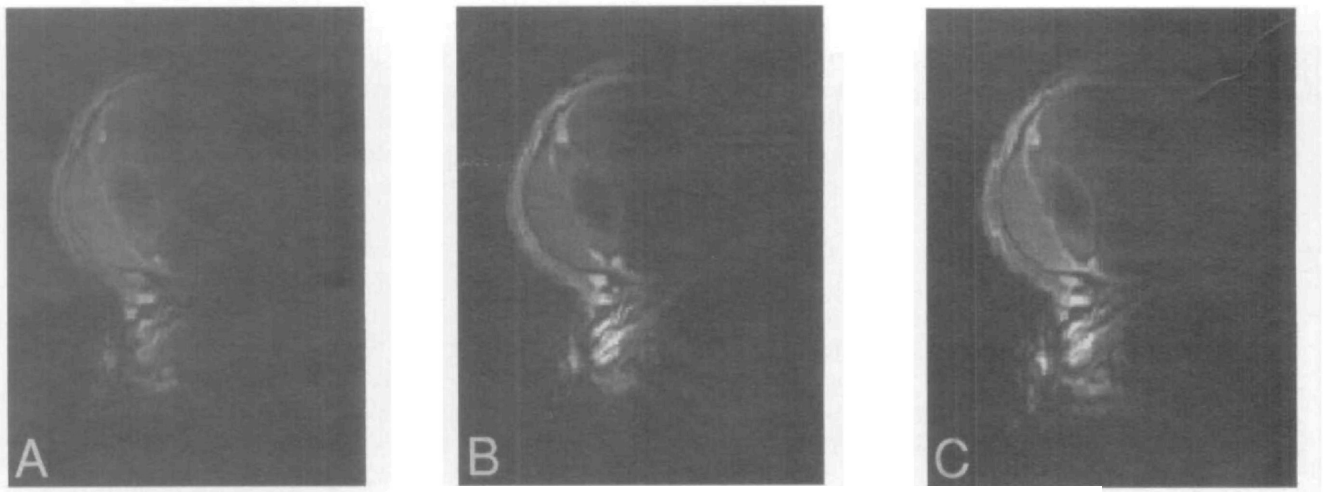

FIGURE 3. ^{1}H magnetic resonance images of a monkey eye (**A**) before, (**B**) 3 minutes after, and (**C**) 33 minutes after the intravenous administration of Gd-DTPA.

through the iris root was first proposed by Raviola,[16] but methods were not available to test the hypothesis. The existence of this pathway has since been demonstrated using fluorosceinated horseradish peroxidase as an intravascular tracer.[1-2,5,15] In vivo aqueous fluorophotometry studies provided kinetic data for the anterior chamber. Tracer studies, performed on animals killed at various time points after the administration of fluorosceinated horseradish peroxidase, provided time-dependent localization data for the structures of the anterior globe. These experimental data, combined with computational models based on the principles of fluid mechanics, were used to describe the diffusion of proteins through the anterior uvea and into the anterior chamber of rabbits and monkeys. All these results suggested an anterior diffusional pathway through which most of the plasma-derived proteins measured in the anterior chamber aqueous humor never enter the posterior chamber. However, no technique existed to verify the hypothesis that plasma-derived tracers were not entering the posterior chamber.

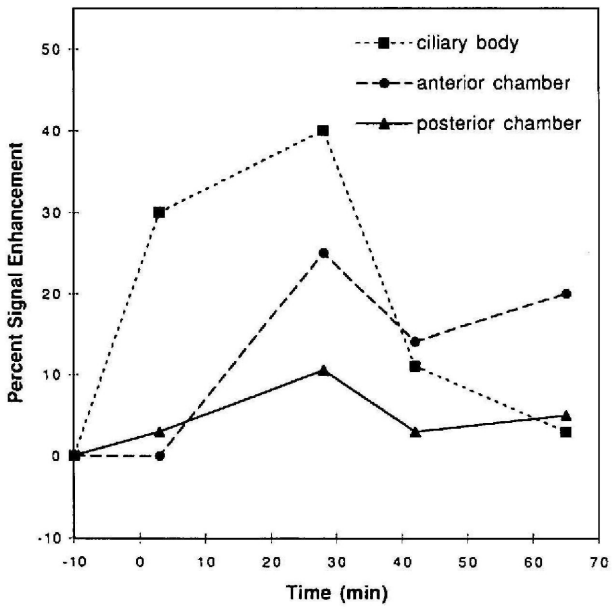

FIGURE 4. Percent signal enhancement, E, as a function of time after the administration of Gd-DTPA (at $t = 0$) for the ciliary body and the anterior and posterior chambers of a monkey eye.

The results of the contrast-enhanced MRI studies described in this article demonstrate unequivocally and noninvasively that the posterior chamber is not involved in the anterior diffusional pathway. Several experimental factors were necessary to reach this conclusion. First, given the dimensions of the ocular structures of interest, it was necessary to obtain well-resolved images to obtain quantitative signal intensity data for each structure. Using a small, sensitive surface coil, combined with a limited field of view and 256 × 256 data points, this was achieved. Sufficient signal-to-noise for these very small regions was obtained by acquiring data over 4 to 5 minutes. Figures 1 and 3 demonstrate the excellent resolution of the various structures of interest in magnetic resonance images of the rabbit and monkey eye.

Second, a concentration-dependent MRI signal enhancement was necessary. The dependence of MRI signal enhancement on Gd-DTPA concentration has been well established by Berkowitz et al[11-14] in their studies of blood–retinal barrier permeability. In these studies, quantitative results were based on the assumption that the enhancement of the MRI signal, E, is proportional to the product of the concentration of the Gd-DTPA, its relaxivity, and the time dependence of the MRI signal. They also demonstrated that the

relaxivity, $T1^{-1}$, is proportional to the concentration of the Gd-DTPA. Because the time dependence of the MRI signal for each tissue is a constant, for a given repetition time, by measuring signal enhancement as a function of time after administration of Gd-DTPA, we can obtain data that depend on the concentration of Gd-DTPA in the tissue.

The lower limit of detection of Gd-DTPA has not been defined and depends on many factors, including magnetic field strength, echo and recycle times in the pulse sequence used to produce the image, and inherent relaxivity of water protons in various tissues in the absence of contrast agent, among others.[12,17] The equation for water proton relaxivity $(T1)^{-1}$ as a function of Gd-DTPA concentration varies, for example, from vitreous, to water, to blood plasma.[12] In their study of the blood–retinal barrier breakdown, Berkowitz et al[12] estimated the water proton relaxivity to be 4.0 1 $mmol^{-1}$ sec^{-1}. For these reasons, the detection threshold of Gd-DTPA is a less definable parameter than would be the detection limit of a fluorophor by fluorophotometric methods. Even though we cannot point to a threshold detection value of Gd-DTPA, however, we can state with confidence that the relative enhancements of the anterior chamber and ciliary body are fivefold or greater than those of the posterior chamber. This is apparent visually in Figure 1, in which a series of magnetic resonance images of a rabbit eye obtained at various times before and after the administration of Gd-DTPA displays increasing signal intensity in the ciliary body and the anterior chamber, as well as constant intensity in the posterior chamber.

Because Gd-DTPA is smaller than proteins and does not bind plasma proteins,[18,19] it might pass through barriers inaccessible to these larger molecules, although this has not been observed in previous studies of the blood–retinal barrier.[12] This strengthens the conclusions reached in the current study. Because we were unable to detect Gd-DTPA in the posterior chamber, it seems unlikely that any significant amount of plasma-derived protein passes this way. From previous studies on the blood–retinal and blood–brain barriers, the movement of Gd-DTPA in such tissues is widely accepted to occur by restricted diffusion[12] and not by active transport. Even if Gd-DTPA were to enter the posterior chamber and immediately be transported back to the ciliary body stroma, the flux would have to be very small or the transport transients very fast for amounts that are detectable with these methods not to have accumulated during the current study or during any previous study of intact blood–tissue barriers in an array of species.

The respective time courses of the entrance of Gd-DTPA into the ciliary body and anterior chamber of the rabbit and monkey eye are shown in Figures 2 and 4. They are consistent with the earlier computer modeling predictions about the diffusion of plasma-derived proteins,[1,2] though with different time constants resulting from the much lower molecular weight of Gd-DTPA. The increases in signal enhancement for the ciliary processes and anterior chamber of the monkey follow a slower time course and are smaller than those for similar structures in the rabbit. This result is consistent with those of fluorophotometry studies, in which the data suggest that protein diffusion from the ciliary body to the anterior chamber is much more impeded in monkeys than in rabbits,[2] which is, in turn, consistent with lower normal levels of protein in aqueous humor of primates than of rabbits. The additional critical result from the MRI studies is the lack of enhancement of the posterior chamber, supporting the prediction that protein diffusion into the anterior chamber does not involve the posterior chamber.

Although it is now clear that an anterior pathway exists and that a substantial majority of the plasma-derived aqueous proteins enter by this route, this does not represent all the protein in aqueous humor. Given that the spectrum of proteins in aqueous and plasma are not identical, certain selected proteins are almost certainly moved by active transport; one such candidate might be transferrin. Lens proteins rarely enter the aqueous humor. In addition, resident cells produce certain immunoregulatory proteins, such as TGF-β. These small, but important, additional contributions of protein could be below our detection threshold.

Further experiments to verify the diffusional pathway for plasma-derived proteins from the ciliary body to the anterior chamber will involve the use of magnetic resonance contrast agents of molecular weights similar to those of proteins. We expect these experiments to provide kinetic data more comparable to those of actual proteins. However, we are confident that, because Gd-DTPA failed to enter the posterior chamber, contrast agents of higher molecular weight will behave similarly.

Key Words

aqueous humor, blood–ocular barrier, ciliary body, magnetic resonance imaging, posterior chamber, rabbit eye

Acknowledgments

The authors thank L. Bollinger for the construction of the prototype surface coil used in these experiments (National Institutes of Health grant RR-02305). They also thank William Ryan of Boston University Medical Center and Heidi Chang of Wellesley College for providing crucial assistance in the animal handling and data analysis aspects of this work.

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