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Articles

Quantitative Magnetic Resonance Imaging in Assessment of the Blood-Retinal Barrier

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Application of magnetic resonance imaging (MRI) in two-dimensional quantitative assessment of blood-retinal barrier dysfunction was investigated in rabbits using a 0.1 T (4.25 MHz) resistive system. Reliable and consistent measurements of vitreous T1 were obtained repeatedly, in slices of width 5 mm and X-Y resolution of 1.2 mm. Calibration of reduction of T1 in eyes after injection of gadolinium-DTPA (Gd-DTPA) was performed, resulting in a dose-related response of relaxation rate (1/T1) to the dose of Gd-DTPA injected. Follow-up scans of injected eyes demonstrated a gradual spread of the T1 "hot-spot" as the contrast agent diffused through the vitreous. T1 rose gradually to basal levels by 72 hr. No local effect of Gd-DTPA was found by ophthalmoscopy. Xenon arc photocoagulation of rabbit retina reduced T1 from 1638 ± 54 (n = 6, mean ± SD) ms to 1408 ± 118 (n = 4) msec ($P < 0.01$) throughout the vitreous 5–7 hr after treatment. In treated rabbits receiving 1.0 mmol/l Gd-DTPA intravenously, T1 adjacent to lesions 90–120 min after injection was further reduced in a 63 μl voxel to 670 ± 50 ms (mean ± SD, n = 5) with a minimum pixel value of 285 ± 52 ms. It was estimated that this represented leakage into vitreous of 8.3 nmol Gd-DTPA. Plasma Gd-DTPA concentrations declined rapidly, with half-life of 20–40 min. The findings indicate that MRI is a technique with the potential for repeated quantitative three-dimensional assessment of blood-retinal barrier dysfunction. Invest Ophthalmol Vis Sci 29:663–670, 1988

The initial lesions of diabetic retinopathy include retinal capillary pericyte loss, endothelial cell proliferation, and microaneurysm formation.[1] These changes are accompanied by capillary dilatation and increased capillary permeability due to disruption of the inner blood-retinal barrier. While qualitative increases in permeability are demonstrated readily by fluorescein angiography, quantifying fluorescein leakage by vitreous fluorophotometry continues to be subject to several practical problems. These include the age-related reduction in light transmission by the lens, the necessity for clear optic media,[2–4] an intact vitreous gel[5] and the restriction with current fluorophotometers (eg, the Fluorotron Master, Coherent Radiation, Palo Alto, CA) to assessment of leakage in one dimension from the perimacular area of retina anteriorly (ref. 6, and Plehwe WE, unpublished data). In the absence of differential spectrofluorophotometry, it must be assumed that the transport rates of free (non-protein-bound) fluorescein and its major metabolite (fluorescein glucuronide) into, and out of the vitreous, are identical.[7,8]

Since many of the lesions of diabetic retinopathy are observed in the mid-periphery, and may be comparatively severe in the presence of relatively mild disease in the macular field,[9] application of a technique with three-dimensional capability such as magnetic resonance imaging (MRI) provided an opportunity for more complete assessment of blood-retinal barrier dysfunction in individual cases.

MRI has been used to examine the anatomy and pathology of the eye, and very encouraging images with contrast and spatial resolution equivalent to, and in some cases superior in quality to those of early X-ray CT scanners have been obtained.[10–16] Appropriate selection of scanning parameters has allowed areas of particular interest to be emphasized, with suggestions of blood-retinal barrier dysfunction in some cases[15,16] but in few studies only has quantita-

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tive determination of altered relaxation times (T1 [spin-lattice or longitudinal relaxation time] and T2 [spin-spin or transverse relaxation time]) been attempted.[17] The relaxation times on proton scanning of vitreous are extremely long, reflecting its high content of water (98.5%[18]). By direct analogy to the use of fluorescein during vitreous fluorophotometry, the availability of contrast agents for use in association with MRI afforded the possibility of measuring blood-retinal barrier dysfunction in the whole eye. Of the contrast agents which have been described,[19] paramagnetic substances, which alter the local magnetic environment (eg, gadolinium) have the potential to enhance proton relaxation and thereby shorten T1 and T2. While it is possible to produce T2-weighted images in vivo, direct quantitative measurement of long T2 times with the magnetic resonance scanners currently available is extremely difficult technically.

This study was undertaken in order to explore the feasibility of the use of MRI as an investigational technique for quantifying the disruption of the blood-retinal barrier(s), by measurement of the relaxation time, T1, in selected volumes of rabbit vitreous in vivo after administration of gadolinium. In the present study, quantitative measurement of T2 was not attempted.

Materials and Methods

Animals

Adult pigmented rabbits (2.9–4.5 kg) were used exclusively in this study. For all ocular manipulations, animals were sedated with intramuscular fentanyl/fluanisone (Hypnorm, Crown Chemical Co., Lamberhurst, England), 0.4 ml/kg, followed by 2–6 mg/kg intravenous pentobarbitone (Sagatal, May and Baker, Dagenham, England). Where necessary, pupils were dilated with 1% cyclopentolate HCl and 10% phenylephrine HCl (Smith and Nephew Pharmaceuticals, Romford, England). All investigations described in this paper were carried out in accordance with the ARVO Resolution on the Use of Animals in Research.

Magnetic Resonance Imaging

Scans were performed by the two-dimensional Fourier transform technique at 4.25 MHz on a 0.1 tesla resistive system, which has been described previously.[20] Contiguous 5 mm multi-slice scans were performed using saturation recovery with TR = 500 ms and field echo acquisition. For the quantitation of T1, single-slice (width 5 mm) images were generated using inversion recovery with TR = 3000 ms and TI = 1000 ms, and saturation recovery with TR = 2000 ms. For each voxel, the signal intensity of the inversion recovery scan was divided by that of the saturation recovery scan and the computed T1 value for this quotient was obtained from tables of T1, derived from standard equations.[21] The receiver was a three-turn surface spiral coil, wound from 5 mm solid copper wire, with a final outer diameter of 75 mm and an inner diameter of 25 mm, aligned with its axis perpendicular to the axis z, of the magnetic field, B_o. The receiver coil remained in a fixed position to avoid possible interaction with the transmitter coil. Scan resolution was 128 × 128 pixels over a 150 × 150 mm field of view. A glass vial containing a solution of gadolinium [III] chloride, $GdCl_3$, with T1 of 1600 msec at 25°C was placed adjacent to the coil to act as an internal quantitative standard. Any scan in which T1 of the standard differed by more than 10% from its true value was not used for quantitative purposes. The accuracy of computed T1 was assessed over the range 300–2000 ms using agarose gels calibrated in a Bruker (Coventry, UK) minispec pc 8 spectrometer operating at 8 MHz.[21]

Effect of Gadolinium on Vitreous Relaxation Times

To measure the efficacy of gadolinium as a contrast agent in MRI of the eye, 0.25–1.0 μmole of gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) in 50 μl, diluted in 0.9% normal saline, was injected directly through the pars plana into the mid-vitreous, and MRI performed 5–6 hr later. Vitreous was not aspirated prior to injection, and eyes were examined by indirect ophthalmoscopy to exclude retinal artery or vein occlusion. This compound was used in preference to $GdCl_3$, due to the high toxicity of the inorganic compound. This time period was selected initially to allow diffusion of the agent in order to try to achieve a uniform concentration of Gd-DTPA in the mid-vitreous. Eyes were examined by indirect ophthalmoscopy at daily intervals for the appearance of any localized reaction to the contrast agent. Following the initial scans, MRI was repeated at 24, 48 and 72 hr after injection of 1.0 μmole Gd-DTPA to determine the fate of the contrast agent in the vitreous.

Disruption of the Blood-Retinal Barrier

Lesions in the blood-retinal barrier were created in deeply anesthetized animals by the application of 12 (n = 2 rabbits) or 40 (n = 9) xenon arc photocoagulation burns (O'Malley Light Coagulator, Clinitex Inc, Danvers, MA; power setting 2, duration 1.0 second, pupil diaphragm 8 mm diameter). In order to allow ready distinction between treated and normal retina, and to preserve the animals' vision, burns were

placed in a group either above or below the posterior pole of the eye. At 4–6 hr after recovery from anesthesia, rabbits were given 0.25 (n = 2) or 1.0 (n = 5) mmole/kg Gd-DTPA intravenously, and were reanesthetized prior to performing MRI at 90–120 min. To ensure survival, it was found necessary to allow animals to recover from the initial anesthesia prior to administration of the large volume (2.0 ml/kg) of hypertonic solution (500 mmole/l) of Gd-DTPA. Four rabbits did not receive Gd-DTPA after photocoagulation, and acted as controls.

To assess the time course of Gd-DTPA clearance from the circulation such that an indication of the optimal time for scanning could be obtained, blood was sampled from two rabbits at intervals until the completion of MRI, and T1 was measured in plasma in vitro in a Bruker minispec pc 8 benchtop spectrometer operating at 8 MHz and 25°C. Standards were prepared by addition of known amounts of Gd-DTPA to rabbit plasma.

Statistical Methods

The significance of differences between groups was assessed by student unpaired t-test. Data from one eye only for each animal were used.

Results

Calibration and Validation of T1 Measurements

Calibration of the system over the range 300–2000 msec using agarose gels indicated that 95% confidence limits for computed T1 were within 10% of the true value throughout this range (Fig. 1).

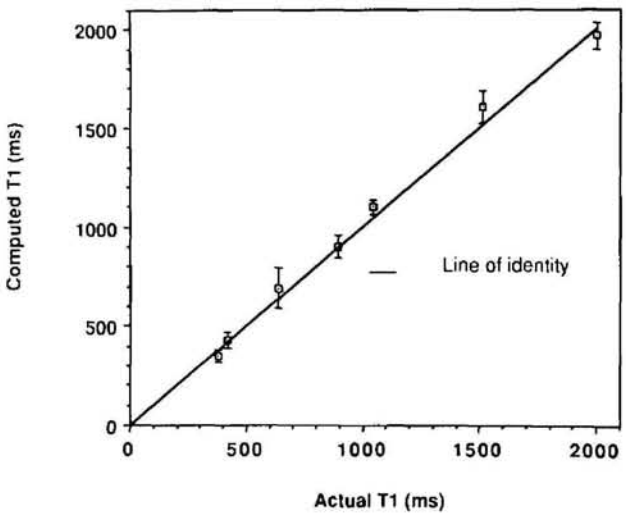

Fig. 1. Calibration of 0.1 t resistive system as used for T1 over the range 300–2000 msec, using agarose gels as standards. Values are given as 95% confidence intervals.

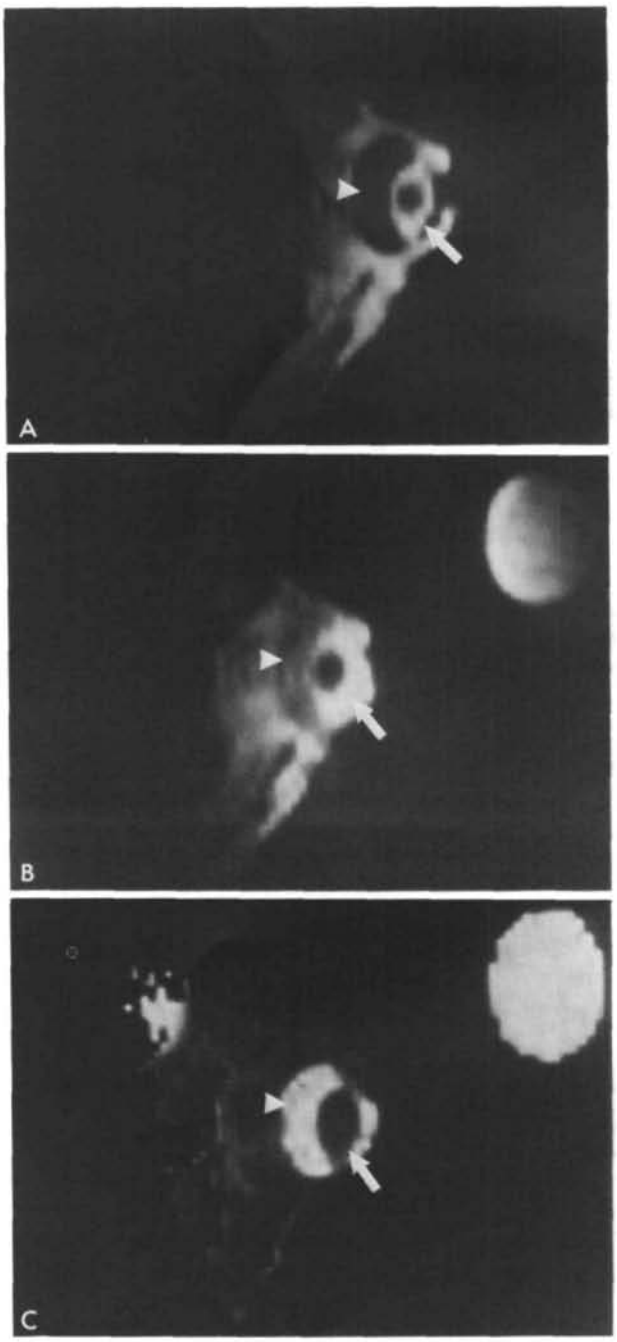

Fig. 2. (A) Representative inversion recovery (IR) scan of a control eye. (B) Representative saturation recovery (SR) scan of the control eye shown in (A). (C) Computed T1 image derived from scans (A) and (B). The circular object (upper right) in the SR scan and computed T1 image is the internal standard. In each image, the arrow indicates the lens and the arrowhead indicates the vitreous.

Effect of Gd-DTPA Injection into Rabbit Vitreous

Inversion recovery scans of control eyes without Gd-DTPA showed the posterior vitreous clearly demarcated from the retina-choroid-sclera and extraocular tissues (Fig. 2A). Similarly, the lens bordered the anterior vitreous, and was itself distinguished from

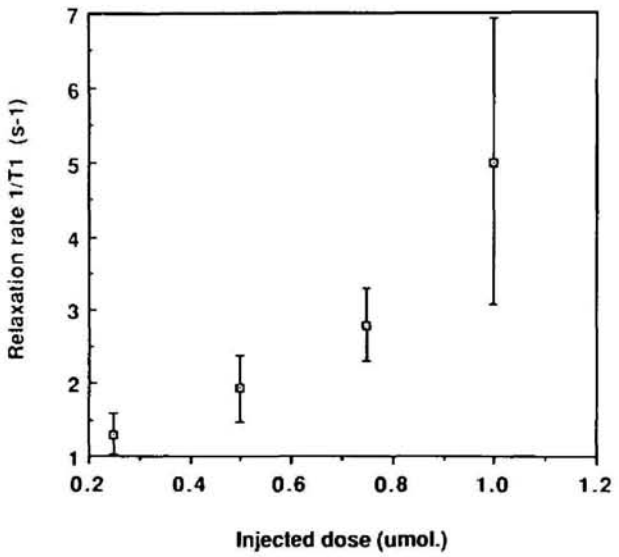

Fig. 3. Relationship between dose of Gd-DTPA injected directly into the rabbit vitreous, and the relaxation rate (1/T1). Values are given as 95% confidence intervals.

the anterior chamber. Computed T1 in the vitreous was 1638 ± 54 ms (mean ± S.D., n = 6), and changed abruptly at the posterior vitreous boundary to 545 ± 17 ms (n = 5) in the region corresponding to the extraocular tissues. Representative saturation recovery and computed T1 scans are shown in Figure 2B and C.

Intravitreal injection of 0.25–1.0 μmole Gd-DTPA in 50 μl did not cause retinal vascular occlusion in any eye. Inversion recovery scans of these eyes 5–6 hr after injection demonstrated a white area in the mid-vitreous, gradually fading to black at the periphery of the vitreous. These white areas corresponded to a reduction of T1 to 180 ± 81 ms (mean ± S.D.) ms (n = 3) for the maximum dose. The response curve of relaxation rate (1/T1) to dose of Gd-DTPA injected is shown in Figure 3. The approximate half-maximal response in relaxation rate occurred at 0.6 μmole Gd-DTPA. A representative inversion recovery scan and its computed T1 image are shown in Figure 4A and B. Repeated examination of injected eyes by indirect ophthalmoscopy revealed no macroscopic reaction to the administered contrast agent.

Temporal Response of Vitreous T1 After Gd-DTPA Injection

Repeated MRI (inversion recovery and saturation recovery scans) of eyes which had received 1.0 μmole Gd-DTPA showed a progressive increase in computed T1 values, achieving basal levels by 72 hr (Fig. 5). The initial "hot-spot" corresponding to the localized volume of Gd-DTPA spread gradually, presumably due to diffusion of Gd-DTPA throughout the vitreous.

T1 Changes After Disruption of the Blood-Retinal Barrier

In four control rabbits treated with 40 xenon arc photocoagulation burns in the lower half of the posterior pole, T1 of the vitreous 7 hr after treatment was reduced significantly to 1408 ± 118 ms ($P < 0.01$), and a gradient was found in one, rising from 1161 ms adjacent to the lesions to normal (1603 ms) at the vitreous base superoanteriorly. The appearances of the IR scan and the computed T1 image remained unchanged from those of untreated eyes. In two rabbits, MRI was performed at 72 hr after photocoagulation, and T1 values were again normal (1630, 1650 ms). In preliminary experiments, two rabbits were given 0.25 mmole/kg Gd-DTPA 24 hr after application of 12 xenon arc photocoagulation burns, and MRI performed 6 hr thereafter. However, results were equivocal, and five rabbits were then treated with 40 photocoagulation burns, and 6 hr later re-

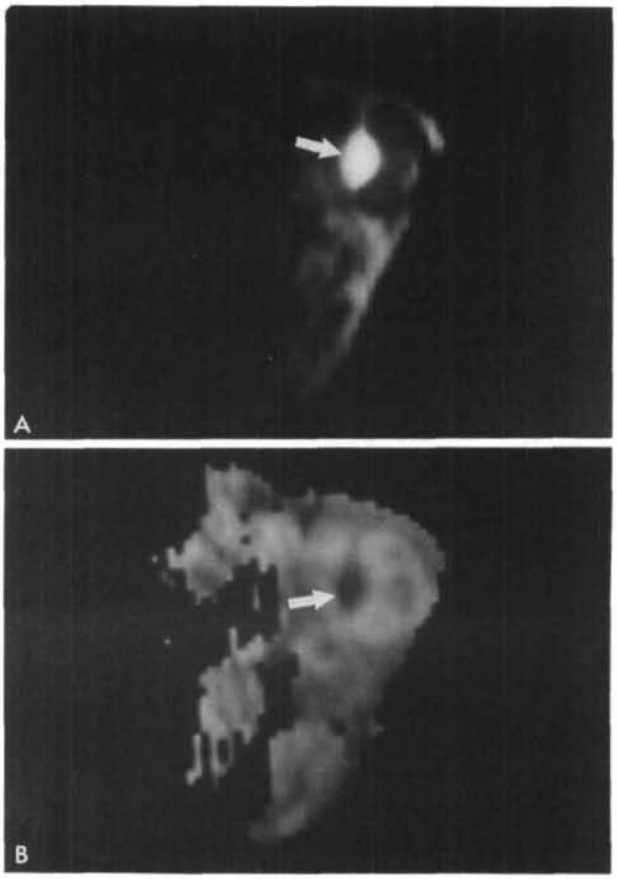

Fig. 4. (A) IR scan of rabbit eye 4 hr after direct injection of 0.5 μmole Gd-DTPA in 50 μl. (B) Computed T1 image derived from scan (A). In each image, the arrow indicates the region of reduced T1 corresponding to injected Gd-DTPA.

ceived 1.0 mmole/kg Gd-DTPA intravenously. MRI performed at 90–120 min revealed leakage of contrast agent through the treated area of retina, appearing as a bright white concavity in the normal black vitreous on inversion recovery scans. Computed T1 in a nine pixel (63 μl voxel) region of interest was 670 ± 50 ms, with a mean minimum pixel value in this region of 285 ± 52 ms. The mean computed T1 in vitreous away from the region of obvious leakage with extremely low T1 was 1460 ± 60 ms. A representative inversion recovery scan and its computed T1 image are shown in Figure 6A and B.

Plasma T1 in Rabbits Receiving Gd-DTPA

Relaxation rate (1/T1) increased linearly in vitro with the concentration of Gd-DTPA to 5 mmol/l in plasma (Fig. 7). This permitted estimates of the concentration of circulating Gd-DTPA to be made, as relaxation rate declined during the 2 hr of study (Fig. 8). The estimated half-life was 20–40 min, but was not constant over time.

Discussion

In order to allow the use of MRI in assessments of the integrity of the blood-retinal barrier, reliability and consistency of the T1 data had to be demonstrated. This was done in two ways. Initially, the machine was calibrated with agarose gels of which the T1 had been determined independently, and T1 data were found to be consistent and reliable over the required range, almost to T1 of water (2650 ms at 25°C). Second, the use of the internal standard in scans ensured that any data obtained could be used confidently. Applicability of the system with the imaging sequences as designated (for long T1 and long T2) was then assessed in the rabbit in vivo and long T1 values, consistent with the high water content, were obtained repeatedly for rabbit vitreous. This finding opened the possibility of measuring blood-retinal barrier dysfunction over the entire retinal surface by systemic administration of a paramagnetic substance which would enhance vitreous water proton relaxation after leakage through the barrier in a manner analogous to measurement of fluorescein in vitreous fluorophotometry. It may not be possible to extrapolate these data directly to eyes of other species, in which vitreous collagen content may be quite different (eg, bovine eyes).

In order to use Gd-DTPA in a manner analogous to fluorescein, calibration of T1 changes with known amounts of Gd-DTPA injected directly into the eye was performed. The volume of Gd-DTPA solution injected in each case (50 μl) was insufficient to cause

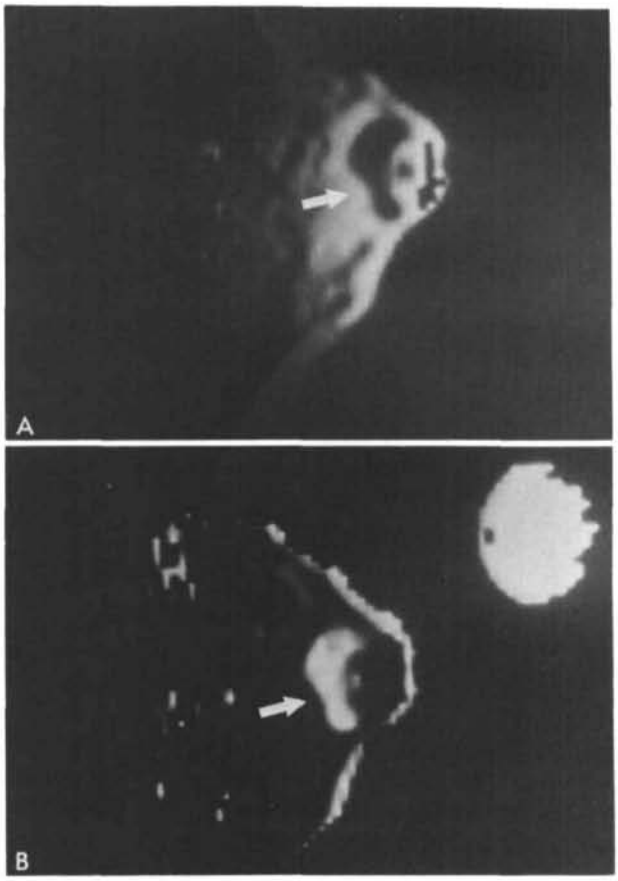

Fig. 6. (**A**) Representative IR scan of a rabbit eye 120 min after intravenous injection of 1.0 mmole/kg Gd-DTPA. The eye was treated 7 hr earlier with 40 photocoagulation burns applied to the lower half of the posterior pole in the region of reduced T1 indicated by the arrow. (**B**) Computed T1 image derived from scan (A).

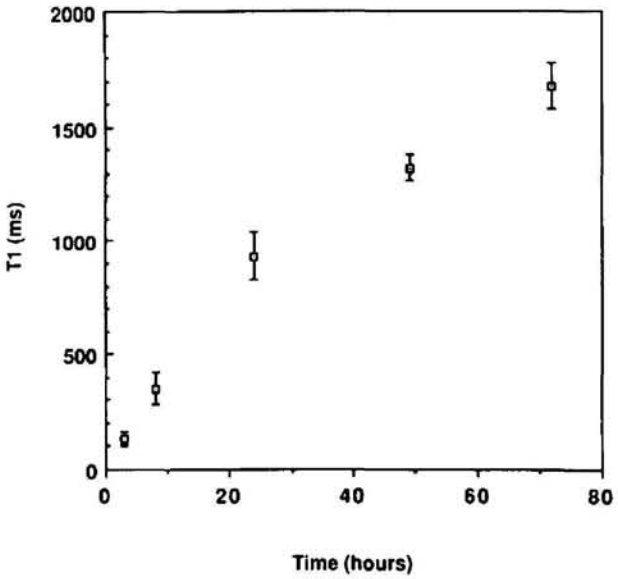

Fig. 5. Temporal response of T1 in eyes which received 1.0 μmole Gd-DTPA by direct injection at time = 0 hr.

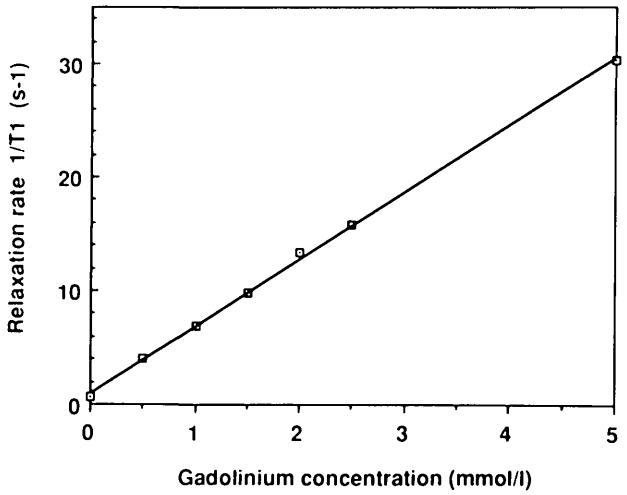

Fig. 7. Relaxation rate (1/T1) as a function of concentration of Gd-DTPA in rabbit plasma.

retinal artery or vein occlusion, and itself changed the volume of vitreous by less than 5%.[22] However, intraocular pressure was not measured directly. Over the range of Gd-DTPA injected (0.25–1.0 μmole), dose-related response of relaxation rate (1/T1) to Gd-DTPA was obtained, allowing quantitative estimates of Gd-DTPA leakage into the vitreous to be made, in situations where the background T1 was normal.

Repeated scanning of eyes which had received the largest dose showed a gradual spread of the reduced T1 "hot-spot" presumably due to diffusion of Gd-DTPA throughout the vitreous, and a homogeneous image with uniform T1 was present 24 hr after injection. Follow-up after longer time intervals demonstrated a gradual increase of T1, achieving basal levels by 72 hr. This is similar to the time course of the loss of fluorescein from the eye, which is in part transported actively. The molecular weight of Gd-DTPA (550 Da[23]) is similar to that of the chief metabolite of

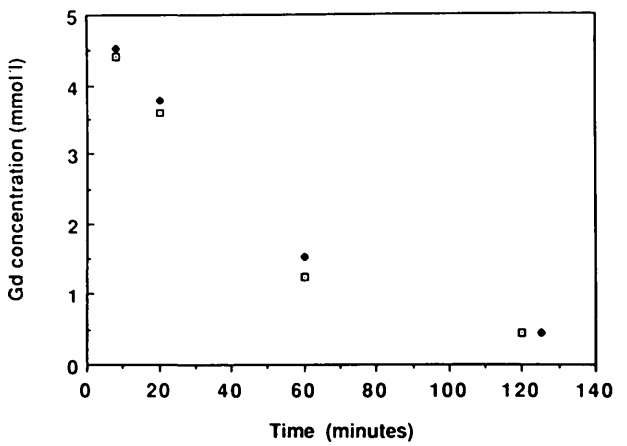

Fig. 8. Concentration of Gd-DTPA, derived from relaxation rate (1/T1) (Fig. 7) in plasma of two rabbits as a function of time after intravenous injection of 1.0 mmole/kg Gd-DTPA.

fluorescein, fluorescein monoglucuronide (508 Da[24]), but on a structural basis, it is unlikely that Gd-DTPA is transported by a similar carrier. Further investigations are required to determine the mode of egress of this agent from the eye. However, if this time course also applies in the human eye, Gd-DTPA could be used repeatedly for assessments of blood-retinal barrier dysfunction. Whether other, even more stable gadolinium complexes are removed similarly (eg, gadolinium cryptelates[25] remains to be determined. Kinetics of Gd-DTPA in the vitreous would most likely be affected by posterior vitreous detachment or syneresis of vitreous, as is the case with fluorescein; however, the effect of these alterations was not investigated in this study. Repeated ophthalmoscopic examination of injected eyes did not reveal any localized macroscopic reaction to Gd-DTPA. Subtle biochemical or biophysical alterations in retinal function were not excluded, and further, more detailed studies may be required to exclude possible toxic local effects.

Measurement of vitreous T1 in eyes that had undergone photocoagulation 5–8 hr previously indicated a reduction of approximately 14% from basal values, which indicated that in any clinical studies of blood-retinal barrier dysfunction or of uveitis, the basal T1 of vitreous must be taken into consideration. Three days after photocoagulation, T1 values were again normal. This reduction of T1 may be a consequence of raised protein concentration locally in the vitreous, after leakage across the disrupted retina, and improvement of this leakage by 3 days may explain the normal T1 values obtained at this time. An effect of shortened relaxation times attributable to protein leakage was described qualitatively in cases of uveal melanoma,[15] but would be difficult to quantify directly without the use of a contrast agent such as gadolinium, of which the specific activity in reducing T1 is known or may be calculated.

In contrast, in those animals scanned 90–120 min after receiving Gd-DTPA intravenously, T1 was reduced by up to 80% immediately adjacent to the area of photocoagulation, with rising values as distance from the lesions increased. T1 values anteriorly, and on the opposite side of the globe, were normal or near normal. Since photocoagulation reduced vitreous T1 significantly, the calibration data obtained previously could not be applied directly to calculate leakage of Gd-DTPA into the vitreous. From theoretical considerations,[26] it was possible to calculate the amount of Gd-DTPA entering the vitreous, and to compare this with fluorescein concentrations obtained in rabbits by vitreous fluorophotometry. In considering the effect of a paramagnetic substance (concentration M), with q hydration sites and affecting the relaxation of water protons:

$$\frac{1}{T1obs} = \frac{1}{T1free} + \frac{qM}{NT1m} \quad (1)$$

T1obs is T1 in the presence of the paramagnetic substance, T1free is T1 in the absence of the paramagnetic substance, N is the concentration of water, and T1m is the relaxation constant for protons in water molecules in close proximity to gadolinium and has a value of 12.3 μsec at 4 MHz (derived from ref. 27).

In the case of Gd-DTPA approaching water molecules in vitreous, q = 4, and if the vitreous content of water is 98.5%,[17] N = 55.0 mol/l. Vitreous T1free after photocoagulation, prior to administration of Gd-DTPA was 1408 ms, and T1obs (after Gd-DTPA injection) was 670 ms. It follows that in 63 μl, the concentration of Gd-DTPA was 132 μmol/l, with a total of 8.3 nmol Gd-DTPA detected.

In rabbits treated with xenon arc photocoagulation, and given 14 mg/kg (37 μmole/kg) sodium fluorescein, homogeneous concentrations of fluorescent material equivalent to 50–100 ng/ml fluorescein were detectable by vitreous fluorophotometry 6 hr after intravenous administration of fluorescein (Plehwe WE, et al, unpublished data). Since approximately two-thirds of this material is present as fluorescein glucuronide,[8] of which the fluorescence is 18% that of fluorescein, the estimated amount of fluorescein and fluorescein glucuronide in a rabbit vitreous of 1.3 ml volume under these conditions is 0.4–0.8 nmol. However the dose of Gd-DTPA given was 27-fold higher than the dose of fluorescein, and assuming that in this model both free and protein-bound fluorescein may enter the vitreous, a total of 11–22 nmol of fluorescein and its glucuronide might be present in the eye, if the higher dose were given. This estimate is comparable to the amount of Gd-DTPA detected (8.3 nmol).

From the data presented and from theoretical considerations, it is clear that the sensitivity of MRI in detection of Gd-DTPA by the reduction of vitreous T1 is considerably lower than that for the detection of fluorescent material by vitreous fluorophotometry, in which concentrations equivalent to 2 ng/ml (5 nmol/l) fluorescein may be distinguished reliably from zero. With the equipment used for MRI, a reduction in T1 of at least two standard deviations (108 ms) from the mean of normal, corresponding to 6.77 μmol/l Gd-DTPA would be required, indicating a 1200-fold lower sensitivity at the lower limit of detection. The half-maximal response in vitreous fluorophotometry is obtained at approximately 500 ng/ml (1.33 μmol/l). Using equation (1) above, 50% reduction in T1 occurs with 103.5 μmol/l Gd-DTPA, suggesting a rightward shift of the dose-response curve in comparison with that of fluorescein by a factor of 78. Improved resolution with apparatus of higher magnetic field strength may allow even better consistency in results to be obtained, and increase the sensitivity of detection of the contrast agent at very low concentrations.

By examination of the dynamic changes of T1 in plasma, the rate of disappearance of Gd-DTPA was comparable to the estimates of others in rats[23] and to the rate of disappearance of free (non-protein-bound) fluorescein.[28] Since circulating concentrations of Gd-DTPA 2 hr after injection were approximately 10% of the initial concentration, it seems that little or no additional information would be obtained by increasing the time between administration of the agent and commencing MRI. Whether the time interval selected (90–120 min) is optimal has not yet been determined.

The xenon arc photocoagulation model used to examine effects of retinal disruption in this study causes a major, although temporary disruption of the retina, which is not directly comparable with the chronic dysfunction of the blood-retinal barrier in clinical conditions such as diabetic retinopathy, in which sensitive quantitative diagnosis is required. Improved sensitivity of the technique may allow its application in other experimental models, and eventually its introduction in clinical assessments.

In summary, the use of MRI in assessment of blood-retinal barrier dysfunction has been investigated. This technique offers at least two potential advantages over the traditional method, vitreous fluorophotometry, in that assessments may be made in at least two dimensions rather than one, and the method does not require the presence of clear optic media. The contrast agent used in this study, Gd-DTPA, is removed rapidly from the rabbit eye, and offers the potential for the procedure to be repeated at intervals. At present, MRI is a relatively lengthy procedure, and its sensitivity in detection of the contrast agent used remains considerably lower than the sensitivity for detection of fluorescein by vitreous fluorophotometry. Further work is required to realize the full potential of the technique.

Key words: magnetic resonance imaging, T1 measurement, gadolinium-DTPA, blood-retinal barrier, rabbit

Acknowledgments

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