Accurate and Precise Measurement of Blood-Retinal Barrier Breakdown Using Dynamic Gd-DTPA MRI

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Dynamic T₁-weighted magnetic resonance imaging (MRI) after the injection of Gd-DTPA is a promising method for investigating breakdown of the blood-retinal barrier (BRB). Previously, the authors demonstrated that in a T₁-weighted image, the initial rate of change in the vitreous water MRI signal as gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) enters the vitreous space strongly correlated with the extent of BRB breakdown. Here, a practical approach to measuring a more relevant physiologic parameter is presented: the permeability surface area product (PS). The theory is a development of earlier work used in investigating the breakdown of the blood-brain barrier. The accuracy and precision of this approach was investigated in rabbits pretreated with sodium iodate (30 mg/kg intravenously). The MRI-derived PS normalized to the area of leaky retina (5.65 ± 0.25 × 10⁻⁴ cm/min, mean ± standard error of the mean; n = 6) was compared to a similarly normalized PS calculated using a classical physiologic method (4.12 ± 0.73 × 10⁻⁴ cm/min; n = 6). Good agreement between the two methods was found (P = 0.09). This result demonstrates that the MRI-derived PS is an accurate and precise measure of BRB breakdown under these conditions. The mathematical model of Gd-DTPA distribution in vivo also is validated. Based on these results, several potential sources of error are discussed, including the effect of back-flow of Gd-DTPA from the vitreous space to the plasma, the underlying vascular patency, and MRI slice selection. Invest Ophthalmol Vis Sci 33:3500–3506, 1992

Recently, we demonstrated that dynamic, T₁-weighted magnetic resonance imaging (MRI) after injection of the magnetic contrast agent gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) is a promising method for assessing breakdown of the blood-retinal barrier (BRB) in the rabbit eye in vivo.¹ When the BRB is disrupted, Gd-DTPA enters the vitreous space and increases the surrounding water proton relaxation rate (e.g., [T₁]⁻¹) in direct proportion to the Gd-DTPA concentration. As previously reported, the rate of change in the vitreous water proton signal after injection correlates with the degree of BRB breakdown and relates to the permeability surface area product (PS) of the lesion.¹

A method for measuring PS from the initial part of the enhancement curve recently was described.² The enhancement curve results from plotting the signal in a T₁-weighted sequence versus time after injection of Gd-DTPA. The theory is a development of earlier work used in investigating the breakdown of the blood-brain barrier.³ Application of this approach was performed on simulated MRI data of cerebral multiple sclerosis and retinal lesions.² However, direct comparison between the MRI method and traditional physiologic methods of measuring PS have not been performed. In the present study, after sodium iodate breakdown of the outer BRB in the rabbit eye in vivo, we first demonstrate the application of the “Simplified Early Enhancement” method of Tofts and Berkowitz² to measure PS of the retina. Second, we investigate the accuracy (systematic error) and precision (reproducibility) of the PS derived from the MRI data by comparing it to the PS obtained, on the same eyes, using a classical physiologic method.

Materials and Methods

Animal Preparation

The 2–3 kg male or female mini-lop rabbits used in this study were treated in accordance with Institutional guidelines and the ARVO Resolution on the Use of Animals in Research. Twenty four hours before the experiment, conscious animals were restrained in a rabbit box and given a bolus of 30 mg/kg sodium iodate via the marginal ear vein. On the day of
the experiment, the animals were anesthetized with a mixture of ketamine HCl (35 mg/kg) and xylazine HCl (5 mg/kg) intramuscularly. Anesthesia was maintained by intravenous infusion of ketamine (20–40 mg/kg/hr) and xylazine (1 mg/kg/hr) via the auricular vein. The heart rate and blood pressure were continually monitored from a femoral arterial catheter, as previously described. The animal was artificially ventilated via an endotracheal tube. Blood gas monitoring (micro 13; Instrumentation Laboratories, Lexington, MA) was performed periodically to ensure proper status of the animal. The rectal temperature of the animal was maintained at 37–38°C via a circulating water blanket connected to a constant temperature bath. The animal was gently placed in a home-built nonmagnetic cradle and secured. At the end of the experiments, the animals were killed by KCl injection intravenously.

**Magnetic Resonance Imaging Procedure**

All experiments were performed on a 4.7 T GE CSI horizontal bore system (General Electric, Freemont, CA) using a whole head, on-edge, split capacitance Helmholtz coil (diameter 9 cm) tuned to 200 MHz. The animal was positioned so that a single coronal slice went through the center of both eyes and was oriented perpendicular to the myelin wing. Data were collected using a standard spin echo sequence with TR = 450 ms and TE = 28 ms, and a 3 mm slice thickness. The field of view varied between 60 × 60 mm and 85 × 85 mm, depending on the head size. Images were obtained with 128 phase encode steps, 256 complex data points, and two acquisitions per phase encode step. Each image took approximately 3 min to acquire. The experimental protocol consisted of collecting a control image before the introduction of the contrast agent. Equal amounts of Gd-DTPA (0.5 mmol/l/kg; Magnevist; Berlex Labs, Wayne, NJ) and saline then were injected over a 3 min period into the ear vein. Setting the center of the injection as t = 0, images were collected such that the zero phase encode gradient came at t = 3.0, 6.5, 11.5, 16.5, and 21.5 min after injection.

To determine the vitreous T1 in the absence of Gd-DTPA (T10, vide infra) a progressive saturation T1 experiment was performed. In a control rabbit, images were obtained using parameters similar to those already mentioned, but with TR = 450 ms, 900 ms, 2 sec, 6 sec, and 10 sec (in random order). At each TR, the signal intensity from both eyes were averaged together and this average value was fit to a standard T1 equation. The vitreous T10 value was 4.05 sec. In contrast, the vitreous T1 value in vitro was 2.69 ± 0.02 sec (mean ± standard error of the mean; n = 4). This difference may have been due to the in vitro versus in vivo temperature difference (25°C versus 37°C). Image analysis was performed on a Macintosh FX II computer using the program Image (W. Rasband, NIH, version 1.41) and consisted of defining a region of interest (ROI) in one image, obtaining a mean signal intensity over that ROI, and applying that same ROI to the other images in the set. In each eye, the ROI for the vitreous was hand-drawn, conforming to the entire retinal surface and including the complete vitreous space. An external vial (1 cm inner diameter) containing Gd-DTPA-doped water was included in each image and provided a signal intensity standard and the spatial calibration (number of pixels/cm) needed for determining the area of the ROI and length of the leaky portion of retina. The ROI signal intensities normalized to the standard then were used in the calculations. To minimize the contribution of random error, PS at times 11.5, 16.5, and 21.5 min post-injection were averaged together in each animal. At these times, the enhancement was greater than 30% in all animals. Statistical analysis was performed using a paired Student’s t-test with P < 0.05 signifying statistical significance.

To establish the Gd-DTPA concentration plasma time course, blood samples (1 ml) were obtained in a separate experiment in heparinized syringes during the control period, immediately after the Gd-DTPA injection, and 6.5, 11.5, 21.5, 31.5, 41.5, and 61.5 min post-injection. These samples were stored in ice. At the end of the MRI experiments, the samples were centrifuged and the plasma fraction was obtained for NMR analysis. A simple inversion recovery T1 experiment was performed on the water signal of the plasma fraction at 25°C. From the T1 value, the amount and thus concentration of Gd-DTPA was determined from a calibration curve obtained at 25°C in a separate phantom study using plasma from control animals.

The vitreous Gd-DTPA concentration at 60 min post injection also was measured. Each animal examined by MRI was killed with KCl intravenously. The eyes were immediately enucleated and frozen by immersion in liquid nitrogen. Differential tissue thawing allowed the sclera, aqueous humor, iris, and lens to be easily separated from the frozen vitreous. The vitreous sample then was weighed to determine its volume, assuming a density of 1 gm/ml. After it reached room temperature, the water proton T1 was measured for each vitreous sample (vide supra) and the Gd-DTPA concentration was determined from a calibration curve previously obtained in a separate phantom study at room temperature using vitreous from animals that had not received Gd-DTPA. The slope of the calibration curve (Gd-DTPA concentration ver-
sus [T\textsubscript{1}^{-1}) produced the Gd-DTPA relaxivity \(R\_s\) (vide infra). The slope and intercept of the calibration curve did not change if the vitreous was first centrifuged and the formed vitreous fraction was removed. In addition, no significant change in the regression parameters was observed between the pH range of 7.4–8.2.

Theory

Non-MRI theory: The flow of tracer into the vitreous is:

\[
\frac{d(C_v(t)V_v)}{dt} = PSC_p(t)
\]

(1)

where \(V_v\) is the whole vitreous volume (ml), \(C_v\) is the mean Gd-DTPA concentration (mmol/l) in \(V_v\), \(C_p(t)\) is the Gd-DTPA plasma concentration (mmol/l) at time \(t\), and \(PS\) is the permeability surface area product (cm\(^3\)/min). Note that \(PS = kV_v\), where \(k\) is the unidirectional rate constant (min\(^{-1}\)).\(^3\) Also note that equation 1 ignores back-flow of Gd-DTPA from the vitreous to the plasma (ie, \(C_p > C_v\)) during the time of the experiment. This is justified by the results of the present experiments (vide infra).

The Gd-DTPA plasma concentration after an injection at time \(t = 0\) is:

\[
C_p(t) = D \sum_{i=1}^{2} a_i \exp(-m_i t)
\]

(2)

where \(D\) is the Gd-DTPA dose (mmol/kg body weight), \(a_i\) are the Gd-DTPA plasma amplitudes (kg/l), and \(m_i\) is the rate constant of each plasma component (min\(^{-1}\)). Computer simulations demonstrate that when \(t = 0\) is set to the center of injection, an injection length of 3 min or less can be treated as a bolus (data not shown). From equation 1,

\[
PS = C_v(t)V_v \int_0^t (p(t)dt)
\]

(3)

Integrating equation 2, we obtain

\[
PS\_{\text{non-MRI}} = \frac{C_v(t)V_v}{(D \sum_{i=1}^{2} a_i[1 - \exp(-m_i t)])/m_i}.
\]

(4)

Thus, knowing the dose \(D\) of Gd-DTPA administered, the plasma parameters (ie, \(a_i\) and \(m_i\)), \(C_v\) and \(V_v\), we can calculate PS.

In the present case, it is helpful to calculate PS per unit area of leaky retina: \(PS\_{\text{non-MRI}} = PS\_{\text{non-MRI}}/A_{\text{ret}}\). Because sodium iodate completely breaks down the outer BRB,\(^7\) \(A_{\text{ret}} \approx \) whole retinal surface area. We can calculate \(A_{\text{ret}}\) from MRI-measured parameters assuming the retinal surface lies on a sphere, that it has circular symmetry, and that the MRI slice passes through the center of eye and the center of the retina. Then, simple solid geometry shows that

\[
A_{\text{ret}} = 2\pi r^2(1 - \cos(L_{\text{ret}}/2r))
\]

(5)

where \(r\) is the sphere's radius and \(L_{\text{ret}}\) is the length of leaky retina in the slice. The sphere's radius was estimated by assuming that the ROI is a sector of a circle. The radius of the circle (and also the sphere) is \(2A_{\text{ROI}}/L_{\text{ret}}\), where \(A_{\text{ROI}}\) is the area of the ROI. \(A_{\text{ROI}}\) and \(L_{\text{ret}}\) are measured directly from the MRI. To check the MRI-derived radius value, we also determined the radius from the volume of the vitreous (assuming a sphere). However, this assumption is not completely accurate, because in the rabbit the lens occupies a significant fraction of the vitreous volume, which makes the value calculated for the radius smaller than expected. In fact, the radius derived from the vitreous volume was \(\approx 15\%\) smaller than that derived from the MRI data. It should be noted this small error in the radius will produce a 40% error in \(v_v\), which would significantly affect the PS calculation. Thus, only the MRI-determined radius was used to calculate \(A_{\text{ret}}\).

MRI theory: To calculate PS from the MRI data, we used the Simplified Early Enhancement method of Tofts and Berkowitz,\(^2\) which is briefly described here.

Tracer analysis of the compartmental model\(^3\) shows that after the end of injection, the concentration in the vitreous is the sum of three exponentials:

\[
C_v(t) = \sum_{i=1}^{3} b_i \exp(-m_i t)
\]

(6)

where \(b_{1,2} = a_{1,2} k/(m_1 - m_{1,2})\), \(b_3 = -(b_1 + b_2)\), and \(m_3 = k\) (the unidirectional rate constant). From the non-MRI experiments, we measured values of \(k \approx 10^{-3}\) min\(^{-1}\) (vide infra). This permits two important simplifying assumptions: (1) that the leak rate constant is insignificant compared to the plasma rate constants (ie, \(m_3 \ll m_1, m_2\)); and (2) that our measurement time is small compared to \(1/m_3 \approx 16\) hr (ie, \(m_3 t < 1\)). Then

\[
C_v(t) = D k \sum_{i=1}^{2} a_i(1 - \exp(-m_i t))/m_i
\]

(7)

which is equivalent to equation 4 (because \(k = PS/V_v\)). The MRI signal from a spin echo sequence with short echo times is:

\[
S_{\text{sd}}(t) = PD(1 - \exp[-T_R/T_1])
\]

(8)

where PD is the proton density, and \(T_R\) is the repetition time. The relaxation rate \((1/T_1)\) is increased by the presence of Gd-DTPA:

\[
1/T_1 = (1/T_{10}) + R_1 C_v
\]

(9)

where \(T_{10}\) is the \(T_1\) in the absence of Gd-DTPA, and \(R_1\) is the relaxivity of Gd-DTPA (l mmol\(^{-1}\) sec\(^{-1}\)). We define the signal enhancement as the fractional increase in signal in the presence of Gd-DTPA:

\[
A_{\text{ret}} = 2\pi r^2(1 - \cos(L_{\text{ret}}/2r))
\]
E = \frac{(S(t) - S_0)}{S_0} = \frac{1 - \exp(-T_R[R,C_v + 1/T_{10}])}{[1 - \exp(-T_R/T_{10})]} - 1 \tag{10}

where \( S(t) \) is the signal intensity at time \( t \) and \( S_0 \) is the control signal intensity (i.e., before injection of Gd-DTPA). At low Gd-DTPA levels, the enhancement increases linearly with concentration, and

\[
E \approx C_v \frac{dE}{dC_v} = R,T_k C_v \tag{11}
\]

Then, using equations 7 and 11,

\[
k = \frac{E}{(R,T_k D \sum \Delta a [1 - \exp(-m_i t)]/m_i) \tag{12}
\]

Thus, \( k \) can be found from a single measurement of enhancement, provided \( R,T_k, \) and the plasma parameters \( \Delta a \) and \( m_i \) are known. Setting the volume of vitreous in the slice \( V_v = A_{ROI} \times \) (slice thickness), \( PS \) then is:

\[
PS_{MRI} = kA_{ROI} \times \text{(slice thickness)} \tag{13}
\]

This equation assumes that all tracer in the slice originated from the portion of retina in the slice. This assumption will not hold at times after injection when tracer from neighboring slices enters the observed slice. However, we assume that at early time points intra- rather than inter-slice flow of tracer dominates. This assumption is justified by the results of the present experiment (vide infra), which demonstrate that \( PS_{MRI} \) are independent of time for the first 20 min post-injection.

In addition, the PS/unit surface area of leaky retina (where the surface area of leaky retina = \( L_{ret} \times \) (slice thickness)) is:

\[
PS'_{MRI} = kA_{ROI}/L_{ret} \tag{14}
\]

Note that equation 14 is independent of the slice thickness. It is assumed that change in the signal intensity in the slice is derived from the leak in the slice because neighboring leaky sections of retina are equivalent.

Previously, \(^1\) speculation was that the initial rate of change in the MRI signal after injection of Gd-DTPA was related to \( PS \) according the equation (which was incorrectly stated \(^1\)): \( dS_{se}(0)/dt = s(0) + (E F_a/\delta) \), where \( E \) is the extraction fraction of Gd-DTPA from the choroid circulation \( (E = 1 - \exp(-PS/F)) \), \( F \) is the choroidal blood flow (ml/min), \( \delta \) is the initial Gd-DTPA plasma concentration, and \( \delta \) is an unknown local tissue influenced constant. The correct relationship should have read \( dS_{se}(0)/dt = EF_a/\delta \). When \( PS \ll F \), this expression simplifies to \( PS_{se}/\delta \), which is independent of blood flow. This simplification appears justified, because the choroidal blood flow \((\approx 1 \text{ ml/min})\) is much greater than the sodium iodate-induced \( PS \) of \( 10^{-3} - 10^{-4} \text{ cm}^2/\text{min}^9 \) (vide infra). Comparing this relationship with that previously obtained by the initial slope method, \(^2,3\) we find that \( \delta = V_v/(S_0 R,T_k) \).

## Results

### Calibration Curves

Figure 1 illustrates the room temperature calibration curve relating the Gd-DTPA concentration to the water proton \( (T_1)^{-1} \) in samples of vitreous. The linear regression results on data from similar experiments performed in distilled water and plasma are presented in Figure 1. Note that for a leaky BRB, a higher vitreous protein concentration is possible because of contamination from plasma protein. This higher protein concentration can alter \( R,T_k \). \(^1\) To adjust for possible protein contamination, we chose to use a value for \( R,T_k \) midway between the vitreous and plasma values (Fig. 1).

### Plasma Gd-DTPA Time Course

Figure 2 illustrates the Gd-DTPA concentration plasma curve after a 3 min injection of 0.5 mmol/kg intravenously. The Gd-DTPA plasma parameters were obtained by fitting to a biexponential function and are presented in Table 1. It should be noted that previously we presented a similar curve after a 6 min injection of 1 mmol/kg intravenously. \(^1\) However, in that report the concentration axis was incorrectly labelled and those data should not be used in future calculations.

![Fig. 1. Room temperature relaxation rate \((T_1)^{-1}\) versus Gd-DTPA concentration calibration curve in vitreous. A linear regression fit to these data is described by the equation \((T_1)^{-1} = 3.28(\text{Gd-DTPA}) + 0.363 (r^2 = 0.995)\). In addition, the linear regression results from similar experiments performed in distilled water and plasma are \((T_1)^{-1} = 3.6(\text{Gd-DTPA}) + 0.335 (r^2 = 0.999)\) and \(4.35(\text{Gd-DTPA}) + 0.380 (r^2 = 0.999)\), respectively. From these three slopes, we estimated the relaxivity \((R,T_k)\) in this study to be 4.0 \text{ mmol}^{-1} \text{ sec}^{-1}.](https://iovs.arvojournals.org/doi/abs/10.1167/iovs.10-6478)
Table 2. Non-MRI parameters

<table>
<thead>
<tr>
<th>Eye</th>
<th>[Gd-DTPA]*</th>
<th>Aν*</th>
<th>PS'MRI × 10^-4</th>
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<tbody>
<tr>
<td>1 OS</td>
<td>0.202</td>
<td>5.94</td>
<td>6.58</td>
</tr>
<tr>
<td>1 OD</td>
<td>0.191</td>
<td>5.78</td>
<td>6.27</td>
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<tr>
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<tr>
<td>2 OD</td>
<td>0.101</td>
<td>7.91</td>
<td>2.94</td>
</tr>
<tr>
<td>3 OS</td>
<td>0.100</td>
<td>9.66</td>
<td>2.82</td>
</tr>
<tr>
<td>3 OD</td>
<td>0.104</td>
<td>9.65</td>
<td>2.95</td>
</tr>
</tbody>
</table>

* [Gd-DTPA] in vitreous (mmol/l) at t = 61.5 min.
† Area of retina (cm²) calculated using Equation 5.
‡ Normalized PS per unit leaky area of retina (cm/min).

MRI results to those obtained using a classical physiologic technique. Excellent agreement was found for the derived PS between these two methods. In addition, the MRI-derived PS values were nearly constant during the MRI experiment. These results validate the mathematical model of Gd-DTPA distribution in vivo described in this study.

To obtain accurate and precise PS values from the MRI method, potential sources of error in equation 12 must be understood and minimized. For E, error will be minimized in cases of a high signal-to-noise (« 100) and if the signal is normalized to an external standard. For R₁, we estimate an error 10-20%, based on the SEM of the linear regression fit. In addition, R₂ and Tₑ (which is « T₁0) are temperature sensitive; this could introduce error when a calibration curve obtained at a different temperature than that in vivo is used. However, this dependence was not evaluated in this study and will be the subject of future investigations. Although the plasma parameters a₁ and m₁, obtained in this study, reasonably agree with those found in previous studies in rats and humans, evaluating these parameters in a greater number of animals will improve their accuracy. Based on these considerations, we estimate an error in PS'MRI of 17-35%.

A few additional assumptions of the model must be discussed. First, because Gd-DTPA undergoes restricted diffusion, it is important to verify that a concentration gradient from the plasma to the vitreous

Table 3. MRI parameters

<table>
<thead>
<tr>
<th>Eye</th>
<th>E*</th>
<th>A₀b†</th>
<th>Lₘ‡</th>
<th>PS'MRIδ × 10^-4</th>
</tr>
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<tr>
<td>1 OS</td>
<td>0.698</td>
<td>1.32</td>
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<tr>
<td>1 OD</td>
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<td>0.617</td>
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<tr>
<td>2 OD</td>
<td>0.585</td>
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<tr>
<td>3 OS</td>
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<td>4.47</td>
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<tr>
<td>3 OD</td>
<td>0.482</td>
<td>2.12</td>
<td>4.45</td>
<td>5.32</td>
</tr>
</tbody>
</table>

* Mean enhancement factor derived from Equation 10 using E at t = 11.5, 16.5, and 21.5 min.
† Area of ROI (cm²).
‡ Length of leaky retina (cm).
§ Normalized PS per unit area of leaky retina (cm/min). PS's were calculated using the mean enhancement factor in this table.
trations, obtained by averaging the Gd-DTPA concentration measured as 1.7, 1.1, and 0.13 mmol/l compared to the respective plasma concentration gradient from the blood to the vitreous that theoretically, because of the large vitreous sink available immediately sank to be minimal.\textsuperscript{2} To check this, sodium iodate-treated animal. In this animal, enhancements \(> 30\%\) were found at \(t = 6.5\) min. In general, enhancements \(> 30\%\) were always found at \(t = 11.5\) and greater. (B) The average \(\text{PS}_{\text{non-MR}}\) \((n = 6)\) at each time point is presented. During the time course of the MRI experiment, measured \(\text{PS}'\) permeability surface area product normalized to the area of leaky retina was nearly constant. Note that these values are not different \((P = 0.09)\) from the mean \(\text{PS}_{\text{non-MR}}\) \((4.12 \pm 0.73 \times 10^{-4}\) cm/min). existed during the MRI experiment. This gradient prevents back-flow effects from underestimating the derived \(\text{PS}.\) Although the Gd-DTPA concentration in the vitreous near the retina is higher than the average value in the vitreous, we expect this effect to be small, because by 60 min diffusion within the vitreous has removed any large concentration gradients.\textsuperscript{1,10} Theoretically, because of the large vitreous sink available for Gd-DTPA distribution, back-flow effects are expected to be minimal.\textsuperscript{2} To check this, sodium iodate-treated animals \((n = 3)\) were killed at \(t = 6.5, 16.5,\) or 31.5 min and the vitreous Gd-DTPA concentration was measured in vitro. The respective concentrations, obtained by averaging the Gd-DTPA concentration in each eye together, were 0.071, 0.084, and 0.13 mmol/l compared to the respective plasma Gd-DTPA concentration measured as 1.7, 1.1, and 1.0 mmol/l \((\text{Fig. 2})\). Thus, the large Gd-DTPA concentration gradient from the blood to the vitreous that existed during the experiment will minimize possible back-flow effects.

Another assumption of the MRI method concerns the patency of the underlying vascular structure. When the Gd-DTPA plasma curve obtained from a peripheral artery does not reflect the functional form of the Gd-DTPA plasma curve in the retinal vessel or choroid, the accuracy but not the precision of the PS determination may be significantly affected. For example, diode laser photocoagulation of the retina typically produces choroidal thrombosis.\textsuperscript{11} Thus, the evolution of the Gd-DTPA concentration in the plasma at a laser burn lesion is expected to be different than that measured at a peripheral artery. Nonetheless, useful comparison between animals of similar pathophysiology still are readily obtained using this method. Note that the influence of vascular patency on accuracy is inherent in any tracer-based method and not just the MRI method.

In the present study, data from a single thin \((3\) mm) slice was used to derive \(\text{PS}.\) Because the lesion size was much greater than the slice thickness, normalizing the \(\text{PS}\) to unit area leaky retina was necessary to compare the MRI and non-MRI methods. For a small lesion completely within the slice thickness, accurate measure of total \(\text{PS}\) should be possible using equation 11. Generally, however, for a lesion size that is small compared to the slice thickness, the derived \(\text{PS}\) may be inaccurate and imprecise, because the lesion may be missed or only partially observed. In cases where the lesion size and location are known, a somewhat better approach is to measure the total \(\text{PS}\) from a thick slice image centered on the lesion. When the lesion size and location are unknown, collecting a multi-slice or 3 dimensional image set would be the best approach for unequivocally determining lesion location and \(\text{PS}\). In the present study, the above assumptions were satisfied by the sodium iodate-induced BRB breakdown. This model produced uniform destruction of the outer BRB, allowing an estimate of the leaky area of retina for the non-MRI method. Based on the established uniform destruction of the retinal pigment epithelium produced by sodium iodate, we assumed that the \(\text{PS}\) per unit area determined from the MRI method is independent of slice iodate treatment disrupts only the retinal pigment epithelial (RPE) cells' without affecting choroidal blood flow. Thus, the functional form of the Gd-DTPA plasma curve obtained from an ear artery also will describe the evolution of Gd-DTPA in the choroid.

Previously, Ennis and Betz used uniformly radiolabelled sucrose to measure the whole retina \(\text{PS}\) in rats treated with sodium iodate intraperitoneally.\textsuperscript{9} Based

![Fig. 3. (A) Representative enhancement curve after a 0.5 mmol/kg Gd-DTPA injection over 3 min (squares, OD; circles, OS) into a sodium iodate-treated animal. In this animal, enhancements > 30% were found at t = 6.5 min. In general, enhancements > 30% were always found at t = 11.5 and greater. (B) The average PS\textsubscript{non-MRI} (n = 6) at each time point is presented. During the time course of the MRI experiment, measured PS' permeability surface area product normalized to the area of leaky retina was nearly constant. Note that these values are not different (P = 0.09) from the mean PS\textsubscript{non-MRI} (4.12 ± 0.73 × 10\textsuperscript{-4} cm/min).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933384/)
on the somewhat different experimental situations, it might be expected that the PS measured by Ennis and Betz would be similar or perhaps less than that found in the present experiment. Ennis and Betz measured a PS of 9.72 ± 1.86 × 10⁻⁵ cm³/min. In contrast, we measured a PS_{non-MRI} of 4.12 ± 0.73 × 10⁻⁴ cm³/min (assuming that the rabbit retina surface area is ~8 times that of the rat). This difference cannot be explained by different tracer molecular weights, because sucrose and Gd-DTPA have similar molecular weights (342 versus 505, respectively). One possible explanation is that different species were used in each study (rat versus rabbit) and the response of the RPE cells to sodium iodate may be species dependent. However, a more likely explanation may involve the route of administration used in the two studies. In the present report, sodium iodate (30 mg/kg) was given as an intravenous bolus, whereas Ennis and Betz administered the same concentration intraperitoneally. Because drug release into the plasma is slower from intraperitoneal injection compared to an intravenous bolus, we expect a more dilute plasma sodium iodate concentration at any time after intraperitoneal injection rather than after an intravenous bolus. A previous study suggested that the extent of BRB breakdown is related to the sodium iodate dose. Thus, PS measured after intraperitoneal administration of sodium iodate might be expected to be significantly smaller than that measured after intravenous injection.

Quantitative assessment of BRB breakdown traditionally has been performed by vitreous fluorophotometry (VFP). Comparison of the accuracy and precision of VFP and the MRI techniques is facilitated by noting that both methods are examples of a more general approach to quantifying breakdown of the BRB. This approach involves intravenous administration of a tracer, which normally does not penetrate the BRB, followed by tissue sampling and analysis. The general mathematical model for this type of experiment is given by equation 3. A problem with applying equation 3 to quantify the BRB breakdown by VFP is the use of fluorescein as the tracer. Fluorescein is rapidly metabolized in the plasma to another fluorescent compound—fluorescein glucuronide (FG). This can complicate interpretation of the arterial plasma integral in equation 3. In addition, fluorescein and FG are actively transported from the vitreous to the blood supply. This also may introduce error into the numerator. Thus, while VFP data can precisely determine PS, the accuracy of this measurement remains to be determined. In contrast, Gd-DTPA acts as a somewhat more ideal tracer than fluorescein because Gd-DTPA is not metabolized in vivo and does not appear to be actively transported out of the vitreous.

**Key words:** blood-retinal barrier, Gd-DTPA, MRI, permeability

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**References**