Imaging the extracellular pH of tumors by MRI after injection of a single cocktail of $T_1$ and $T_2$ contrast agents

Gary V. Martínez, Xiaomeng Zhang, María L. García-Martín, David L. Morse, Mark Woods, A. Dean Sherry and Robert J. Gillies

The extracellular pH ($pH_e$) of solid tumors is acidic, and there is evidence that this acidity is related to invasiveness. Herein, we describe an MRI single-infusion method to measure $pH_e$ in gliomas using a cocktail of contrast agents (CAs). The cocktail contained gadolinium–1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminophosphonate (GdDOTA-4AmP) and dysprosium–1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraakis(methyleneephosphonic acid) (DyDOTP), whose effects on relaxation are sensitive and insensitive to $pH$, respectively. The Gd-CA dominated the spin–lattice relaxivity $\Delta R_1$, whereas the Dy-CA dominated the spin–spin relaxivity $\Delta R_2$. The $\Delta R_2$ effects were used to determine the pixel-wise concentration of [Dy] which, in turn, was used to calculate a value for [Gd] concentration. This value was used to convert $\Delta R_1$ values to the molar relaxivity $\Delta r$ and, hence, $pH_e$ maps. The development of the method involved in vivo calibration and measurements in a rat brain glioma model. The calibration phase consisted of determining a quantitative relationship between $\Delta R_1$ and $\Delta R_2$ induced by the two $pH$-independent CAs, gadolinium–diethylentriaminepentaacetic acid (GdDTPA) and DyDOTP, using echo planar spectroscopic imaging (EPSI) and $T_1$-weighted images. The intensities and linewidths of the water peaks in EPSI images were affected by CA and were used to follow the pharmacokinetics. These data showed a linear relationship between inner- and outer-sphere relaxation rate constants that were used for CA concentration determination. Nonlinearity in the slope of the relationship was observed and ascribed to variations in vascular permeability. In the $pH_e$ measurement phase, GdDOTA-4AmP was infused instead of GdDTPA, and relaxivities were obtained through the combination of interleaved $T_1$-weighted images ($R_1$) and EPSI for $\Delta R_2$. The resulting $r_1$ values yielded $pH_e$ maps with high spatial resolution. Copyright © 2011 John Wiley & Sons, Ltd.

**Keywords:** pH; susceptibility; contrast agent; cocktail

INTRODUCTION

The extracellular pH ($pH_e$) of tumors is acidic, and there is evidence that this acidity stimulates local invasion (1,2) and plays a role in metastases (3). This low $pH_e$ is a product of elevated metabolism combined with perfusion limitations. To fully characterize the nature and anatomical extent of this aspect of the altered tumor microenvironment, there is a need for further advances in the ability to accurately map $pH_e$ at high spatial resolution. Furthermore, noninvasive measures of $pH_e$ have been limited to preclinical animal models and there is a real need to develop methods that may eventually be used in humans.

Various approaches have been developed to measure $pH_e$ maps in tumors with MRI and MRSI (4,5). Advances have involved low-resolution, single-voxel $^{31}$P MRS to medium resolution $pH_e$ maps using $^{19}$F-, $^1$H- or $^{13}$C-labeled tracers (6–9). However, methods exist to obtain $pH_e$ maps with a spatial resolution that is on the same scale as MRI, such as magnetization transfer methods (10–12) or $pH$-sensitive relaxivity agents (13). Relaxivity-based methods offer the advantage of lower radiofrequency energy deposition, which is a significant consideration in the clinical setting. However, a challenge in relaxometric methods is the determination of contrast agent (CA) concentration, which is required to convert relaxation data to $pH_e$ maps. Methods to date have used sequential injections of pH-insensitive and pH-sensitive contrast agents, which have used the combination of inter- and outer-sphere relaxivity $Dy$ and, hence, $pH_e$ maps. These authors contributed equally to this work.
pH-sensitive agents (14,15). Although these can provide high spatial resolution maps, they lack high temporal resolution and would be impractical in a clinical setting.

Numerous MRI applications require the determination of CA concentrations, such as dynamic contrast-enhanced MRI. Historically, this has been achieved with either $T_1$-weighted (dynamic contrast) or $T_2^*$-weighted (dynamic susceptibility) pulse sequences (16–18). $T_1$ sequences yield contrast and enhancement based on inner-sphere relaxation, whereas $T_2^*$ contrast is based on outer-sphere mechanisms. Although it is slower, $^1$H echo planar spectroscopic imaging (EPSI) can be highly linear with CA concentration and has been used to measure spectra in both water-suppressed (19–21) and nonwater-suppressed (22,23) modes. One of the advantages of using EPSI in vivo is the ability to determine $R_2$ (or $R_2^*$) and $\Delta R_0$ simultaneously (24). In addition, one may examine the complexity of the lineshape to reveal underlying subvoxel heterogeneities.

In the current work, we investigated the use of EPSI to measure simultaneously the pH-dependent inner-sphere effects and the pH-independent outer-sphere effects on $R_2^*$ that were pH independent with negligible effects on $T_1$. The entire protocol is shown in Fig. 1 and involved the measurement of $[\text{Dy-CA}]$ through its effects on $R_2^*$, and subsequent extrapolation of $[\text{Gd-CA}]$ from the known ratio of $[\text{Dy-CA}]$ to $[\text{Gd-CA}]$. This relationship assumed that Gd-CA and Dy-CA were distributed to the tumors with identical pharmacokinetics, which has been evaluated previously by Raghunand and coworkers (14,15). In this article, we made the assumption that DyDOTP was the same pharmacokinetic profile as GdDOTP as they have similar chemical charge or mass. The calculated $[\text{Gd-CA}]$ was then used to yield the spin–lattice molar relaxivity ($r_1$) and, hence, a pH map was generated based on an in vitro relaxivity titration curve. We have investigated these relationships in vivo in a pharmacokinetic time series in intracranial rat gliomas, which is the first application of a CA cocktail single-infusion method for the determination of spin–lattice and susceptibility-induced transverse relaxation rates.

**MATERIALS AND METHODS**

**Cell lines and in vivo tumor model**

C6 glioma cells ($10^5$) were stereotactically injected into female Wistar rats in the right caudate nucleus to a depth of 5.5 mm ($n = 18$). Tumor growth was monitored with MRI to determine when the gliomas were of a substantial volume of approximately 50% of the right hemisphere. Typically, the tumors were mature enough to observe enhancement from 10 to 14 days after injection.
Data acquisition
Experiments were performed with a Bruker Biospec 4.7-T MRI scanner (Bruker BioSpin MRI GmbH, Ettlingen, Germany), with a pre-emphasis unit. The gradients are capable of 20 mT/m strength, with a maximum slew rate of 110 T/m/s. A volume coil (inside diameter, 73 mm) was used for excitation, and a surface coil (diameter, 18 mm) was used for signal reception. The surface coil was placed 1–2 mm above the rat brain, and active decoupling was used to switch between transmit and receive coils.

In vitro measurements were carried out on 96-well plates cut in half. GdDOTA-4AmP, DyDOTP, and GdDTPA (DTPA, diethylene-triaminepentaacetic acid) were characterized at various concentrations and pH values in order to determine a titration curve between T1 and pH. Both phosphate-buffered saline and fetal bovine serum were used as buffers for relaxation studies. Spin–lattice relaxation measurements were performed in imaging mode to determine spatially localized T1 values in each well of the plate. A region of interest (ROI) was drawn for each well, where the mean value was used to generate a titration curve.

For the pH-sensitive CA, in vitro relaxation experiments were accomplished with variable GdDOTA-4AmP concentrations and pH values. Inversion recovery or progressive saturation experiments were performed to determine a parametric T1 value. After confirming agreement between the two, only progressive saturation was performed. The data were fitted to a three-parameter equation, \( I(t_0) = M(1 - W e^{-T1/T}) \), where \( W \) is the inversion (saturation) parameter and \( t_0 \) is the variable delay after the initial 180° inversion pulse or the repetition time in the case of progressive saturation.

In vivo T1-weighted images were scanned with \( TR = 200 \) ms and \( TE = 6.4 \) ms. Proton density images were acquired with \( TE = 6.4 \) ms and \( TR = 7000 \) ms. T2-weighted images were acquired using a rapid acquisition with relaxation enhancement (RARE) pulse sequence with a RARE factor of eight, giving an effective \( TE = 72 \) ms. Fat suppression was not used. The EPSI experiments were performed with a single radiofrequency excitation pulse and trapezoidal echo planar readout (26), where \( TE = 5 \) ms. The receiver bandwidth was 123–152 kHz, resulting in a spectroscopic bandwidth of 1.2–1.5 ppm. The data were collected with a matrix size of \( 128 \times 128 \times 128 \). The resulting in-plane resolution for all of the imaging and spectroscopic imaging experiments was 250 \( \mu m/pt \) with a field of view of 3.5 cm on each side. A slice thickness of 1.25 mm was used throughout.

The isotropic spectroscopic resolution was 1.8–2.3 Hz/pt.

MR experimental structure
Once the animal had been placed in the magnet and under anesthesia, a series of pre-infusion images and spectroscopic images was acquired. These included scouting fast low-angle shot, RARE axial and coronal, proton density, T1-weighted spin echo and EPSI. As a CA cocktail consisting of Magnevist : DyDOTP\(^{5-}\) in a 1:2 ratio was gradually infused, an interleaved series of two separate pulse sequences, T1-weighted images and EPSI was collected (20–90 min). The rate of intravenous infusion was 0.2–0.4 mmol/kg/h (0.4–0.8 mmol/kg/h for DyDOTP), although the infusion rate was reduced when sufficient enhancement was achieved. In a subset of calibration animals, Carr–Purcell–Meiboom–Gill (CPMG) sequence was interleaved with T1-weighted and EPSI scans. The parameters used for the CPMG sequence were \( TR = 3 \) s, \( TE = 10.5 \) ms, a matrix size of \( 128 \times 128 \times 128 \) echoes. The CPMG sequence was not run on all animals. Instead, a correlation between \( R_2 \) and \( R_2^* \) was used to estimate \( R_2 \) values.

In the pH measurements, the same sequence of experiments was acquired but without CPMG. The CA cocktail in this case was GdDOTA-4AmP\(^3-\) : DyDOTP\(^3-\) (1:2). The infusion was performed as mentioned above.

Data processing and analysis
The reconstruction of T1-weighted images, proton density images and EPSI spectroscopic images was performed using in-house programs written in MATLAB (The MathWorks, Inc., Natick, MA, USA). All other images were reconstructed in ParaVision (Bruker BioSpin MRI GmbH). T1-weighted images were reconstructed by direct inverse fast Fourier transform of the k-space data in two dimensions. Volumes (V) of the tumors were obtained by measuring the area (A) of the tumor in a single slice and using \( V = 4A^{3/2}/3\pi^{1/2} \). EPSI datasets were processed by reducing the data in the spectroscopic dimension into odd echoes and even echoes. This yielded two separate three-dimensional datasets that were independently apodized with a Hanning filter in the k-space and a decaying exponential in the time domain; however, the even-echo planar echoes were time reversed preceding apodization. Reconstruction was accomplished with a two-dimensional inverse fast Fourier transform and a one-dimensional fast Fourier transform to yield two spatial and one spectroscopic dimension. Zero-order autophasing was performed along the spectroscopic dimension. Finally, the odd-echo and even-echo experiments were added together to form a final dataset.

The quantity \( \nu_{\text{FWHM}} \) is the full width at half-maximum of the water peak and is directly proportional to \( R_2^* \). \( \nu_{\text{FWHM}} \) was determined by fitting EPSI spectra with a Lorentzian lineshape on a pixel-by-pixel basis. \( R_2^* \) was calculated directly from the linewidth (LW) according to: \( R_2^* = \pi \times \text{LW} \). \( R_2^* \) values were obtained by a two-parameter fit of the pixel intensity in the CPMG experiment to the equation \( I(TE) = I_0 e^{-TE/R_2^*} \), where \( I_0 \) is the proton density. Smoothing of all datasets and parameter maps was accomplished using a 5 × 5 convolution kernel with a center value of four (the rest were unity).

Calculation of pH
The spin–lattice relaxation rate constant \( R_1 \) was determined, as in previous work (14,15), from the proton density and T1-weighted images, so that its measurement was accomplished throughout the course of the infusion with high temporal resolution. In the current work, we have empirically determined that TE is not much less than T2. However, T2 was incorporated into the repetition time-dependent signal equation to compensate for the apparent saturation of the intensity at higher concentrations of CA:

\[
I(TE) = I_0(1 - e^{-T1/R1})e^{-TE/R2^*}
\]  

where \( I \) is the T1-weighted intensity at any time, \( I_0 \) is the intensity of the proton density image, TR is the repetition time and TE is the echo time. Rearranging Equation [1], we obtain the relaxation rate constant \( R_1 \):

\[
R_1 = -\ln[1 - I/(I_0 e^{-TE/R2^*})]/TR
\]
The relaxation rate is a function of [CA] and is given by:

\[ R_1 = R_{1,0} + r_1[CA] \]  \[3\]

where \( r_1 \) is the spin–lattice molar relaxivity (mM\(^{-1}\) s\(^{-1}\)).

This rearranges to a form in which we can experimentally obtain \( r_1 \) given that we have \( R_1, R_{1,0} \) and [CA]:

\[ r_1 = (R_1 - R_{1,0})/[CA] \]  \[4\]

Combining Equations [2] and [4], the in vivo CA concentration is given by:

\[ [CA] = -\frac{1}{TR_1} \ln \left( \frac{1 - I_{\text{Post}}/(I_0 e^{-TE R_2})}{1 - I_{\text{Pre}}/(I_0 e^{-TE R_2})} \right) \]  \[5\]

where \( I_0, I_{\text{Pre}} \) and \( I_{\text{Post}} \) are the proton density, pre-infusion \( T_1 \)-weighted signal and post-infusion \( T_1 \)-weighted signal, respectively. A calibration was established between the two quantities \( \Delta R_2^* \) and [Gd] for a CA cocktail of two pH-insensitive agents GdDTPA/DyDOTP (1 : 2). It should be noted that in vivo calibration was chosen because the \( R_2^* \) effect is highly dependent on the presence of mesoscopic field inhomogeneities in the tissue of interest \((27,28)\). \( R_2^* \) was determined independently of \( R_1 \) by measuring the linewidth of the water peak in an EPSI experiment. The calibration that was obtained is applicable to the current study assuming that GdDOTA-4AmP and DyDOTP both perfuse and extravasate equivalently, which is based on the fact that these two molecules are of a similar size and have identical charges at physiological pH values. This assumption then yields a linear relationship between [GdDOTA-4AmP] and \( \Delta R_2^* \).

The relaxometric pH\(_e\) imaging method requires a knowledge of \( R_1 \) to yield pH\(_e\). Data from in vitro phantoms (fetal bovine serum) were fitted using a Hill-modified Henderson–Hasselbach equation for multiple titratable groups. In Fig. 2a, a nonlinear least-squares fitting routine was used to fit the following relation for pH\(_e\):

\[ \text{pH} = pK_a - \log_{10} \left( \frac{(r_1 - r_{1,\text{base}})}{(r_{1,\text{acid}} - r_1)} \right)^n \]  \[6\]

Overall, the \( T_1 \)-weighted experiment yields the relaxation rate through Equation [2], and EPSI yields [GdDOTA-4AmP] from the water linewidths. Based on the apparent linear relationship between the tumor volume and the slope of the relationship between concentration and \( \Delta R_2^* \) \((p = 2.2 \times 10^{-4})\), a slope was determined in the pH measurement phase (with zero offset) in order to calculate the concentration. The resulting values were combined to yield the spin–lattice molar relaxivity. Based on Equation [4] and a knowledge of the relaxivity, pH\(_e\) was determined through Equation [6] as outlined in Fig. 1. In the pH map, the ROI fraction is the number of pixels in the tumor ROI that gives a pH value divided by the total number of pixels in the ROI.

**Calculation of error**

The overall error in the pH values was determined by propagating the error of individual measurements according to the error

---

**Figure 2.** (a) In vitro relaxation titration of GdDOTA-4AmP (DOTA-4AmP, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminophosphonate) fitted to a Hill-modified Henderson–Hasselbach equation, (b) the corresponding total pH error as a function of \( r_1 \) and \( \sigma_{r_1}, \) and (c) the error propagation analysis scheme, giving rise to (b). It is based on Equation [4], where the rounded rectangles represent the numerator and rectangles represent the denominator, and Equation [6], where the five rounded shapes in the third row represent the five fitted parameters.
propagation equation (29). In images, the standard deviation of an ROI outside of the image was used. The calculation of the error in \( r_1 \) in each point in the time series was accomplished by including all error contributions in quadrature according to the variances in the independent variables in a numerical fashion, rather than using individual analytical error propagation formulae. As the mean value of \( r_1 \), averaged over various time points in the series (10–30 min), was used to calculate the pH, a mean-weighted error was used to calculate the error in \( r_1 \). However, for the final error in pH as a result of the various parameters whilst varying covariances were not included in the error calculations. The points in the series (10–30 min), was used to calculate the pH, a

\[
\sigma_{\text{pH}} = \sigma_{pK_a} + \sigma_n^2 \left( \log_{10} \left( \frac{\left(r_1 - r_1,\text{base} \right)}{\left(r_1,\text{acid} - r_1 \right)} \right) \right)^2
\]

\[
+ \sigma_{r_{1,\text{acid}}}^2 \left( n \left( \frac{\left(r_1,\text{acid} - r_1 \right)\ln10}{} \right) \right)^2
\]

\[
+ \sigma_{r_{1,\text{base}}}^2 \left( n \left( \frac{\left(r_1 - r_1,\text{base} \right)\ln10}{} \right) \right)^2
\]

\[
+ \sigma_{1}^2 \left( n \left( \frac{\left(r_1 - r_1,\text{base} \right)\ln10}{} \right) \right) - 1 \left( \frac{\left(r_1,\text{acid} - r_1 \right)\ln10}{} \right)^2 \] [7]

The error surface in Fig. 2b was constructed using the best-fit parameters and their corresponding errors: \( p_{K_a}, \sigma_{pK_a}, n, \sigma_n, r_{1,\text{acid}}, \sigma_{r_{1,\text{acid}}} \), \( r_{1,\text{base}} \) and \( \sigma_{r_{1,\text{base}}} \). The overall error expression is:

The nonlinear least-squares fit yielded the best-fit parameters and their corresponding errors: \( p_{K_a}, \sigma_{pK_a}, n, \sigma_n, r_{1,\text{acid}}, \sigma_{r_{1,\text{acid}}}, r_{1,\text{base}}, \sigma_{r_{1,\text{base}}} \). The overall error expression is:

\[
\sigma_{\text{pH}} = \sigma_{pK_a} + \sigma_n^2 \left[ \log_{10} \left( \frac{\left(r_1 - r_1,\text{base} \right)}{\left(r_1,\text{acid} - r_1 \right)} \right) \right]^2
\]

\[
+ \sigma_{r_{1,\text{acid}}}^2 \left( n \left( \frac{\left(r_1,\text{acid} - r_1 \right)\ln10}{} \right) \right)^2
\]

\[
+ \sigma_{r_{1,\text{base}}}^2 \left( n \left( \frac{\left(r_1 - r_1,\text{base} \right)\ln10}{} \right) \right)^2
\]

\[
+ \sigma_{1}^2 \left( n \left( \frac{\left(r_1 - r_1,\text{base} \right)\ln10}{} \right) \right) - 1 \left( \frac{\left(r_1,\text{acid} - r_1 \right)\ln10}{} \right)^2 \] [7]

**RESULTS**

**In vitro calibrations**

*In vitro* relaxivities \( r_1 \) and \( r_2 \) for the CAs in this study are summarized in Table 1. Gd showed greater \( r_1 \) and \( r_2 \) relaxivities than Dy. GdDOTP is shown for reference. Spin–lattice relaxation data for GdDOTA-4AmP as a function of pH were fitted to Equation [6] and yielded the parameters, \( r_{1,\text{acid}} = 5.39 \pm 0.27 \), \( r_{1,\text{base}} = 3.68 \pm 0.10 \), \( p_{K_a} = 6.56 \pm 0.15 \) and \( n = 0.88 \pm 0.28 \), where the square of the correlation coefficient \( (\rho^2) \) was 0.99. The fit is shown in Fig. 2a. Figure 2b shows the surface of the total pH error generated by the Hill-modified Henderson–Hasselbach equation as a function of \( r_1 \) and \( r_2 \), where the individual error of all of the fitting parameters were included: \( \sigma_{r_1}, \sigma_{r_2}, \sigma_{pK_a}, \sigma_{r_{1,\text{acid}}}, \sigma_{r_{1,\text{base}}} \). The total pH error was sensitive to \( r_1 \) and \( r_2 \), in that the error was smallest in the part of the titration curve that was steepest, but was also dependent on the value of \( r_2 \). Notably, \( \sigma_{\text{pH}} \) was smallest in the region in which tumor pixels were expected, which corresponds to pH 6–7.

Table 1. Relevant lanthanide contrast agent (CA) relaxivities \( (r_1 \text{ and } r_2) \) in fetal bovine serum performed at 4.7 T and 37°C

<table>
<thead>
<tr>
<th>Contrast agent</th>
<th>( r_1 (\text{mm}^{-1} \text{s}^{-1}) )</th>
<th>( r_2 (\text{mm}^{-1} \text{s}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GdDTPA</td>
<td>2.95</td>
<td>–</td>
</tr>
<tr>
<td>GdDOTP</td>
<td>3.17</td>
<td>4.75</td>
</tr>
<tr>
<td>DyDOTP</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>DTPA, diethylenetriaminepentaacetic acid; DOTP, 1,4,7, 10-tetraazacyclododecane-( N,N',N'',N''' )-tetrakis(methylene-phosphonic acid).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**In vivo studies**

*In vivo* calibration studies were necessary because the effect of CA on \( R_2^* \) could not readily be recapitulated *in vitro*. Calibration experiments were performed with the CA cocktail GdDTPA/DyDOTP (1 : 2). In Fig. 3, the \( T_1 \)-weighted images showed an increase in intensity as a result of infusion of the CA cocktail into the glioma. Comonvert increases in transverse relaxation (\( R_2 \), \( R_2^* \) and \( \Delta R_2^* \)) were also observed in Fig. 3 as a result of CA cocktail infusion.

Figure 4 shows a spectrum from a single EPSI redundant voxel prior to and after infusion of the CA cocktail. CA caused an increase in the linewidth of the peak. These data could be fitted with a Lorentzian lineshape model to yield \( T_2 \)FWHM. The change in \( T_2 \)FWHM was directly proportional to \( \Delta R_2^* \). Thus, fits of these data in each voxel were used to generate parametric maps of \( R_2^* \) and \( \Delta R_2^* \) (Fig. 3).

The analysis of the data was performed where CA breached the blood–brain barrier (within the glioma), in a contralateral ROI (control) and in the jaw muscle (ROIs depicted in Fig. 5a). The glioma showed the largest relative enhancement, the jaw showed intermediate enhancement and the contralateral side showed almost no enhancement (Fig. 5b). This trend was mirrored in the response of the other parameter maps: \( R_2, R_2^*, [\text{GdDTPA}], [\text{DyDOTP}] \) and \( \Delta R_2^* \). The enhancement was largely contained within the glioma. The effect of \( T_2 \) on the intensity of \( T_1 \)-weighted images was significant. If \( T_2 \) was not used to correct the signal equation, there was an apparent saturation of the intensity as the apparent [CA] reached approximately 0.2 mM.

Figures 6 and 7 show data from the same ROIs as depicted in Fig. 5. Figure 6 shows the response of different parameter maps compared with the relative enhancement from \( T_1 \)-weighted images: \( R_2, R_2^*, \Delta R_2^* \) and \([\text{GdDTPA}], [\text{DyDOTP}]\) and \( \Delta R_2^* \). These plots established the linearity of these parameters with relative enhancement. Of particular significance were the cross-correlations of \( R_2 \)-derived parameters (Fig. 6a) and \( R_2^* \)-derived parameters (Fig. 6b, c) with relative enhancement (\( T_1 \)-weighted spin echo) within the glioma. The linearity of the CA concentration and the change in the transverse relaxation rate constant \( \Delta R_2^* \) was fundamental to this method. Given that \( r_1 \) and [CA] were calculated from the same dataset, a linear dependence was expected and was observed. In some experiments, \( R_2 \) was extracted from the CPMG experiment and showed a linear dependence on [CA] in pixels within the glioma. Linearity was also observed for the dependence of \( R_2 \) on \( R_2^* \) in Fig. 6e, and was of particular importance as this relationship was used to estimate \( R_2 \) values in experiments in which the CPMG sequence was not conducted. Further correlations were observed between [GdDTPA] and [DyDOTP] with \( \Delta R_2^* \) in Fig. 6f. The most significant of these was between [GdDTPA], [DyDOTP] cocktail and \( \Delta R_2^* \), as this relationship allowed [CA] to be calculated from EPSI spectroscopic imaging experiments.

The Gd concentration remained relatively low throughout the course of the experiment (<0.1 mM) (Fig. 6). It was consistently observed that, for the first few points in the infusion, small changes in \( \Delta R_2^* \) were observed together with changes in \( r_1 \). The rate of infusion was 500 times slower than the rate of injection during a typical bolus, where changes were expected to be much slower. Therefore, there was no first pass effect in these experiments as the rate was very slow.

The images and parameter maps that were used to determine the relaxivity are summarized in Fig. 7a–f: \( T_1 \)-weighted, proton

density, $R_2$, $R_1$, $\Delta R_2^+$ and [CA] determined from EPSI. That the two observables can be combined to yield an experimental $r_1$, with the use of Equation [4], is demonstrated in Fig. 7g, h, where the numerator was determined from $R_1$ (pre- and post-contrast) and the denominator was calculated from $\Delta R_2^+$.

The variability in the fitting parameters for the relationship between [GdDTPA] and $\Delta R_2^+$ was analyzed for the calibration cohort (Table 2). The correlation coefficient between the tumor volume and slope was 0.91 with a $p$ value of 0.0002, indicating that there was a statistically significant relationship between tumor burden and slope.

The pH measurement was acquired with the single-infusion method. In this case, all of the data were acquired in the presence of GdDOTA-4AmP instead of GdDTPA. The combination of $T_1$-weighted (Fig. 8a) and proton density (Fig. 8b) images was used to determine $R_1$ (Fig. 8c), where the range of CA concentrations in which the relaxivity was constant was 0.02–0.1 mM. Hence, the $R_1$ map was calculated using data when the concentration was in this range. This also corresponds to the linear regime for the response of the relaxation rate $R_1$.

Figure 8d shows the $\Delta R_2^+$ map that was used to obtain the concentration map in Fig. 8e. The maps in Fig. 8c, e were used to calculate the relaxivity in Fig. 8f. The values of relaxivity within the ROI depicted in Fig. 8a were used to generate a histogram in Fig. 8g. These relaxivity values are shown to be within a reasonable range from the pH titration. The values of GdDOTA-4AmP relaxivity are in agreement with phantom measurements of relaxivity (Fig. 2a). Equation [6] is sensitive to pixel dropout, as values outside the limiting relaxivities yielded complex values. The ROI fractions of pixels were calculated, as described in Materials and Methods, and are summarized in Table 3.

From Fig. 8, a pH map was obtained (Fig. 9). The histograms of values within three different gliomas are shown in Fig. 9A, C and E, and are distributed about a mean pH of 6.6. Figures 9B, D and F show histograms of uncertainties in the pH values determined by the propagation of error from $r_1$ values on a pixel-by-pixel basis, where $r_1$ from different time points was used to calculate the uncertainty, which was typically 0.5 or less. Table 3 shows that the mean pH values within the glioma are acidic relative to healthy tissue, and are relatively consistent.

Figure 4. Representative Lorentzian curve fits to a pixel of echo planar spectroscopic imaging (EPSI) data before (a) and at a late stage (b) (~87 min) after contrast agent (CA) infusion. A concomitant drop in intensity is seen in the peak.
DISCUSSION

In vivo calibration

From the array of images and parameter maps in Fig. 3, it is clear that the blood–brain barrier has been breached, allowing CA to extravasate into the extracellular space of the tumor. The most obvious example of this is the T1-weighted enhancement. This was corroborated by R2 and R2*/C3 parameter maps. It is also clear from the images that not much CA entered the brain outside of the glioma, yet enhancement was noticeable in the jaw muscle.

EPSI was chosen for this study because the spatiotemporal resolution is very high for a slow infusion. In addition, EPSI was selected over a multiple gradient echo relaxation experiment because it shows better time resolution along the free induction decay as a result of rapid switching of the gradients in the echo planar readout. It also allowed for the interrogation of the complexity of the water lineshape. The transverse relaxation rate constant R2* is affected by both mesoscopic and macroscopic magnetic field heterogeneity (27,28), as in regions of variable magnetic susceptibility. These two factors affect the relaxation and hence the linewidths. The R2* maps from EPSI demonstrate an increase in linewidth over the time series (Fig. 3). On closer inspection of spectra from individual pixels, it is clear that the CA had a dramatic effect on the width of the line (Fig. 4). It is this direct relationship between [CA] and R2*/C3 that is the essence of the single-infusion method.

The infusion of the CA cocktail was gradual. The response of the relative enhancement, R1 and R2, was linear over the course of the experiment within the glioma (Fig. 5), where [CA] increased at a rate proportional to the total amount infused. The contralateral side showed negligible build-up, whereas the muscle showed some enhancement. Notably, ΔR2* for both the glioma and muscle were on a similar scale over time, whereas the muscle showed less relative enhancement compared with the glioma. This was probably caused by differences in the extracellular volume fractions and relative vascular permeabilities of the two tissues (30,31), where the ΔR2* changes are caused by mesoscopic heterogeneity, which is a scale much smaller than the MRI spatial resolution of 250 µm.

Figure 5. (a) Depiction of three different regions of interest (ROIs) in the head. (b) R1 versus time. (c) R2 versus time. (d) R2*/C3 versus time. (e) [GdDTPA or DyDOTP] versus time. (f) ΔR2* versus time. Glioma (red circles), contralateral side (green squares) and jaw muscle (blue diamonds). DTPA, diethylene-triaminepentaacetic acid; DOTP, 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetrakis(methyleneephosphonic acid).
In the calibration phase of the experiment, the correlation of the imaging parameters with relative enhancement showed a correspondence with inner-sphere relaxation, which is a function of concentration. $R_2$ and $\frac{R_2}{C_3}$ correlations in Fig. 6 show that CPMG and EPSI may be used to determine the concentration of a pH-insensitive CA. However, previous in vitro experiments with CPMG have shown that $R_2$ tracks closely to $R_1$ when using GdDOTA-4AmP. As $R_2^*$ operates on an outer-sphere mechanism, it is independent of $R_1$ in the presence of GdDOTA-4AmP, and may be used to determine the concentration based on the linear response to [CA] in the glioma, as observed in Fig 6 and, in particular, in Fig. 6f. It is clear that, in a rat glioma, $\Delta R_2^*$ yielded useful concentrations based on its relationship with [Gd].

The effects of transcytomeimal exchange on the intensities and spin–lattice relaxation rates have been studied extensively (32,33). The effect of multiple compartments may have profound implications on CA injection, thereby affecting the relaxometric properties of water exchanging between compartments. However, it is important to note that this series of experiments was performed at CA concentrations sufficiently low to remain within the fast exchange limit (32). Thus, the relaxation rates were not significantly affected by transcytomeimal water exchange.

$R_1$–$R_2^*$–permeability surface

The tumor burden showed a linear dependence on the slope of the relationship between $R_1$ and $\Delta R_2^*$ (Table 2). This dependence was statistically significant with a $p$ value of $2.2 \times 10^{-4}$ (correlation value of 0.91). Experiments were conducted several days after the blood–brain barrier had been breached, leading to visual enhancement of the glioma on infusion of CA. The fact that the tumor burden played a role is probably a result of the observation that glioma vascular permeability increases with size (30). In the present study, permeability was not explicitly measured, but it is considered to be the most probable explanation. We can construct a view in which the slope of the line increases as the tumor grows as a result of increased permeability and, hence, decreased $R_2^*$ effect, leading to a useful relationship that is sensitive to $\Delta R_2^*$ versus [CA] slope.

pH measurement

The $r_1$ measurement of Fig. 8 demonstrated that this approach yielded consistent results when using GdDOTA-4AmP, where the range of relaxivity was within the pH titration range (Fig. 2). The relaxivities fell within the range in which the pH could be obtained according to Equation [6].

---

Figure 6. Various parameters for the three different regions of interest (ROIs) in Fig. 5 denoted as glioma (red circles), contralateral side (green squares) and jaw muscle (blue diamonds). Parameters in (a)–(d) are shown versus relative enhancement: (a) $R_2$, (b) $R_2^*$, (c) $\Delta R_2^*$, (d) [GdDTPA/DyDOTP] (1:2), (e) $R_2$ versus $R_1^*$ (CPMG, EPSI). (f) [GdDTPA or DyDOTP] versus $\Delta R_2^*$ ($T_1$-weighted, EPSI). CPMG, Carr–Purcell–Meiboom–Gill; DTPA, diethylenetriaminepentaacetic acid; DOTP, 1,4,7,10-tetraazacyclododecane-$N,N',N'',N'''$-tetrakis(methylene phosphonic acid); EPSI, echo planar spectroscopic imaging.
The pH maps in Fig. 9 report a mean pH value of 6.7 in this tumor, with a range of values from 6.0 to 7.2. The uncertainty in the pH values is typically below the range of pH values in the glioma. The majority of the pixels within the glioma were acidic relative to normal tissue pH. Other tumors have shown a similar mean and distribution of pH values. The standard error of measurement indicated the uncertainty in the mean value. The propagation of error from $r_1$ to the pH measurement showed that the error was typically smaller than the standard deviation of the pH values. This

The pH maps in Fig. 9 report a mean pH value of 6.7 in this tumor, with a range of values from 6.0 to 7.2. The uncertainty in the pH values is typically below the range of pH values in the glioma. The majority of the pixels within the glioma were acidic relative to normal tissue pH. Other tumors have shown a similar mean and distribution of pH values. The standard error of measurement indicated the uncertainty in the mean value. The propagation of error from $r_1$ to the pH measurement showed that the error was typically smaller than the standard deviation of the pH values. This

Figure 7. Calibration data overview: (a) proton density, (b) $T_1$-weighted, (c) $R_2$ map, (d) $R_1$ map (e) $\Delta R_{2}^*$ map; (f) [GdDTPA] from $\Delta R_{2}^*$, (g) $r_1$ map from (d) and (f), and (h) in vivo $r_1$ histogram for glioma region of interest. DTPA, diethylenetriaminepentaacetic acid; DOTP, 1,4,7,10-tetraazacyclododecane-$N,N',N''$-tetrakis(methyleneephosphonic acid).

Table 2. Summary of relevant parameters for the calibration tumors investigated in this study. The enhancement is denoted by strong (S) or medium (M)

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Matrix size</th>
<th>Enhancement</th>
<th>Tumor volume (mm$^3$)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Mean $r_1 \pm SD$ (mm$^{-3}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>128 x 128</td>
<td>S</td>
<td>50.4</td>
<td>0.0059</td>
<td>-0.0145</td>
<td>1.96 ± 4.93</td>
</tr>
<tr>
<td>2</td>
<td>128 x 128</td>
<td>S</td>
<td>124</td>
<td>0.0264</td>
<td>-0.0654</td>
<td>3.04 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>256 x 256</td>
<td>S</td>
<td>73.5</td>
<td>0.0037</td>
<td>0.0111</td>
<td>3.02 ± 0.080</td>
</tr>
<tr>
<td>4</td>
<td>256 x 256</td>
<td>M</td>
<td>100</td>
<td>0.0104</td>
<td>0.0018</td>
<td>3.04 ± 18.9</td>
</tr>
<tr>
<td>5</td>
<td>256 x 256</td>
<td>M</td>
<td>187</td>
<td>0.0255</td>
<td>0.0127</td>
<td>12.3 ± 68.3</td>
</tr>
<tr>
<td>6</td>
<td>256 x 256</td>
<td>M</td>
<td>65.1</td>
<td>0.0066</td>
<td>0.1409</td>
<td>3.22 ± 1.49</td>
</tr>
<tr>
<td>7</td>
<td>128 x 128</td>
<td>S</td>
<td>209</td>
<td>0.0386</td>
<td>-0.0626</td>
<td>3.06 ± 0.12</td>
</tr>
<tr>
<td>8</td>
<td>128 x 128</td>
<td>M</td>
<td>70.8</td>
<td>0.0105</td>
<td>-0.0329</td>
<td>3.06 ± 0.12</td>
</tr>
<tr>
<td>9</td>
<td>128 x 128</td>
<td>S</td>
<td>68.3</td>
<td>0.0099</td>
<td>0.0037</td>
<td>0.098 ± 20.1</td>
</tr>
<tr>
<td>10</td>
<td>128 x 128</td>
<td>M</td>
<td>41.0</td>
<td>0.0077</td>
<td>0.0039</td>
<td>3.4 ± 1.67</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.0101</td>
<td>0.0027</td>
<td></td>
</tr>
</tbody>
</table>

The slope and intercept were determined by the linear relationship between the gadolinium-diyethylenetriaminepentaacetic acid concentration [GdDTPA] and $\Delta R_{2}^*$. The correlation coefficient between the tumor volume and slope is 0.91 with $p = 2.2 \times 10^{-4}$. SD, standard deviation.
tightness in the error distribution is required for meaningful pH values.

The pH could not be reliably measured for some pixels within the central core of the tumor. This was probably a result of necrosis, where the lack of intact cells strongly affected $R_2^*/C_3$, such that it was well below that observed in tissue. Decreased perfusion of CA to the necrotic core may also be responsible. This is an unfortunate drawback of this approach but, nonetheless, can be addressed by obtaining an MRI diffusion map prior to infusion of CA that can assess cellularity, or a dynamic contrast-enhanced MRI map to assess CA perfusion. In any case, the accurate determination of pH in the necrotic core is of little

Figure 8. pH data overview: (a) $T_1$-weighted, (b) proton density, (c) $R_1$ map, (d) $\Delta R_2^*$ map, (e) [GdDOTA-4AmP] from $\Delta R_2^*$, (f) $r_1$ map from (c) and (e), (g) $r_1$ histogram for glioma region of interest. DOTA-4AmP, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminophosphonate; DOTP, 1,4,7,10-tetraazacyclododecane-$N,N,N',N''$-tetrakis(methylene phosphonic acid).

### Table 3. Summary of relevant parameters, including pH values, when infusing GdDOTA-4AmP (DOTA-4AmP, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminophosphonate). The enhancement is denoted by strong (S), medium (M) or weak (W)

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Matrix size</th>
<th>Enhancement</th>
<th>ROI fraction</th>
<th>Tumor volume (mm³)</th>
<th>Slope</th>
<th>Mean pH ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>128 × 128</td>
<td>M</td>
<td>0.47</td>
<td>18.8</td>
<td>0.0030</td>
<td>6.65 ± 0.66</td>
</tr>
<tr>
<td>12</td>
<td>128 × 128</td>
<td>M</td>
<td>0.099</td>
<td>45.3</td>
<td>0.0072</td>
<td>6.32 ± 0.42</td>
</tr>
<tr>
<td>13</td>
<td>128 × 128</td>
<td>M</td>
<td>0.083</td>
<td>71.5</td>
<td>0.0114</td>
<td>6.28 ± 0.54</td>
</tr>
<tr>
<td>14</td>
<td>128 × 128</td>
<td>M</td>
<td>0.18</td>
<td>17.9</td>
<td>0.0028</td>
<td>6.38 ± 0.57</td>
</tr>
<tr>
<td>15</td>
<td>128 × 128</td>
<td>W</td>
<td>0.18</td>
<td>12.2</td>
<td>0.0019</td>
<td>6.41 ± 0.66</td>
</tr>
<tr>
<td>16</td>
<td>128 × 128</td>
<td>M</td>
<td>0.51</td>
<td>27.3</td>
<td>0.0043</td>
<td>6.58 ± 0.66</td>
</tr>
<tr>
<td>17</td>
<td>128 × 128</td>
<td>M</td>
<td>0.8022</td>
<td>26.5</td>
<td>0.0042</td>
<td>6.61 ± 0.55</td>
</tr>
<tr>
<td>18</td>
<td>128 × 128</td>
<td>S</td>
<td>0.8000</td>
<td>22.9</td>
<td>0.0037</td>
<td>6.66 ± 0.57</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.5</td>
</tr>
</tbody>
</table>

The slope is determined from the tumor volume and the relationship between the gadolinium–diethylenetriaminepentaacetic acid concentration [GdTPA] and $\Delta R_2^*$. ROI, region of interest; SD, standard deviation.
interest, as pH plays a role in tumor progression by acidifying the surrounding tissue as it grows. Hence, we are primarily interested in measuring the pH in the viable parts of the tumor. In addition, pixel dropout in the calculation of pH may be adversely affected where \( \sigma_{\text{pH}} \) is large, which is expected to occur close to either of the plateau regions of the titration curve. However, in human gliomas and other tumor types, there may be nonenhancing regions. In such subregions of a tumor, this method will not apply, as the CA will not be able to extravasate into these areas.

Overall, the mean pH value from all of the tumors was 6.5. Most of the tumors fell into a tight range of volumes when compared with the set used for calibration. Likewise, the slopes showed a relatively small range in the pH group. Because of these small ranges, these results could not be used to assess a relationship between tumor burden, permeability and the mean pH value. The approach of using volume as a criterion to localize tumors in the \([\text{CA}]-R_2^*\)-burden space is justified. It yields consistent mean pH values across the cohort. The three-dimensional relationship was useful, yet a greater understanding of the role played by vascular permeability may be obtained with additional work.

The pH maps obtained in this work are consistent with the general view that the tumor microenvironment is acidic, which may confer a competitive advantage to cancer cells. The further study of various tumors and pH-sensitive CAs using this method may provide additional details on cancer invasion and proliferation.

CONCLUSIONS

A single-infusion protocol, consisting of slow infusion of a CA cocktail, yielded high-resolution pH maps of tumors. The current method is based on a calibration between [Gd] derived from \( T_1 \)-weighted spin echo and \( \Delta R_2^* \). The linear correlation is also a function of permeability. The primary advantage of this protocol over previous studies (6,8,9,11,12,14) is the rapidity of generating the pH measurement after the calibration curve has been obtained. The time resolution of these experiments is the fastest thus far for a matrix size of 128 \( \times \) 128. The times required for pre-infusion and infusion give rise to a total time of
approximately 1 h. The measured relaxivity in this method is a hybrid determination from two separate measurements, whereby $R_1$ values are determined from $T_1$-weighted intensities and [Gd] is determined from EPSI linewidths ($\Delta R_2^* \). The linearity in these two separate observable quantities during infusion indicates that the value of $r_1$ is applicable to the determination of $pH_o$ which is the crux of this approach. This high-resolution $pH_e$ map was obtained with a relatively modest concentration of Gd-DOTA-4AmP, which is approximately $0.10–0.20$ mM at 16 min after infusion. This method is capable of yielding $pH_e$ maps within practical times in a clinical setting using faster bolus injections, with the proviso that complete calibration studies are performed.

Acknowledgements

Thanks go to Christy Howison and Brenda Baggett for technical assistance. This work was supported by National Institutes of Health grants SR01CA077575 and RR-02584, and a grant from the Robert A. Welch Foundation (AT-584).

REFERENCES


