Utility Decay Rates of T1-Weighted Magnetic Resonance Imaging Contrast Based on Redox-Sensitive Paramagnetic Nitroxy1 Contrast Agents

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The availability and applicability of the combination of paramagnetic nitroxyl contrast agent and T1-weighted gradient echo (GE)-based dynamic magnetic resonance imaging (MRI) measurement for redox imaging are described. The time course of T1-weighted GE MRI signal intensities according to first-order paramagnetic loss of a nitroxyl contrast agent were simulated for several experimental conditions. The apparent decay rate calculated based on decreasing T1-weighted MRI contrast \((k_{\text{app}})\) can show an approximate value of the original decay rate \((k_{\text{true}})\) discretely given for simulation with suitable experimental parameters. The difference between \(k_{\text{true}}\) and \(k_{\text{app}}\) can be sufficiently small under T1-weighted spoiled gradient echo (SPGR) scan conditions (repetition time\(=75\) ms, echo time\(=3\) ms, and flip angle\(=45^\circ\)), with a conventional redox-sensitive nitroxyl contrast agent, such as 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL) and/or 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-N-oxyl (carbamoyl-PROXYL), and with i.v. doses of below 1.5 \(\mu\)mol/kg b.w. for mice. The results of this simulation suggest that the \(k_{\text{true}}\) of nitroxyl contrast agents can be the primary index of redox status under biological conditions.

**Key words** nitroxyl contrast agent; redox mapping; T1-weighted contrast; magnetic resonance imaging; tissue redox status; tumor imaging

Nitroxyl radicals have been widely used as a free radical probe for electron paramagnetic resonance (EPR) spectroscopy and EPR imaging (EPRI) to investigate in vivo redox conditions.\textsuperscript{1–6} The enzymatic or chemical reduction system reduces nitroxyl radicals to the corresponding hydroxylamine,\textsuperscript{7–12} enabling us to estimate the redox status in vivo. Hypoxic conditions in the tumor,\textsuperscript{13–15} biological reducing agents,\textsuperscript{16,17} and oxidative stresses accompanying the synthesis of superoxide and/or hydroxyl radical\textsuperscript{18–20} can enhance the reduction of nitroxyl radicals in vivo. In contrast, oxidative conditions in a tissue could apparently decrease the in vivo reduction rate of nitroxyl radicals due to the re-oxidation of hydroxylamine to the original nitroxyl radical.\textsuperscript{21}

The feasibility of nitroxyl radicals as T1-enhancing magnetic resonance imaging (MRI) contrast agents has been described since the early 1980s.\textsuperscript{22,23} Since the T1 relaxation of protons can be affected by paramagnetic nitroxyl electron spin, changing the MRI contrast before and after administration of the nitroxyl contrast agent can reflect the amount of nitroxyl contrast agent in addition to providing simultaneous anatomical mapping, similar to Gd\textsuperscript{3+}. Despite a number of pharmacokinetic studies,\textsuperscript{7,24,25} rapid in vivo reduction of the nitroxyl contrast agent was not as useful as an MRI contrast agent in early 1980s.\textsuperscript{26}

At that time, relatively low T1 relaxivity of nitroxyl contrast agents was another problem for obtaining clinically and/or experimentally utilizable T1-contrast enhancement. Recently, high-power magnet MRI instrumentation, such as 4.7 T, with a T1-weighted spoiled gradient echo (SPGR) pulse sequence has enabled us to take a fresh look at the feasibility of employing nitroxyl radicals as MRI contrast agents.\textsuperscript{27–29} The redox status of the tumor and normal tissue can be reflected in the time course of T1 contrast after the injection of a paramagnetic nitroxyl contrast agent to an experimental animal. Gradient echo (GE)-based T1-weighted contrast imaging is fast and is suitable for dynamic imaging, although T1-weighted MRI contrast can not be utilized to quantify the amount of contrast agent directly. The pseudo decay rate calculated based on decreasing T1-weighted MRI contrast can show a similar value to the decay rate obtained by EPR measurement when an identical phantom sample is measured by both methods.\textsuperscript{27,28} In vivo experiments which compared the decay rates of a nitroxyl contrast agent between normal and tumor tissues of a mouse also showed similar results between EPRI and MRI experiments.\textsuperscript{27,28}

Several problems on EPRI, such as lack of anatomical information, low spatial resolution, low temporal resolution, and relatively small sample volume, can be solved using MRI; however, the pseudo decay rate obtained based on T1-weighted MRI contrast is not a theoretically true decay rate because the detection of nitroxyl radical using MRI is indirect, based on the enhancement of T1 contrast instead of the direct detection of radio frequency (RF) absorption. Therefore, the theoretical reliability of the pseudo decay rate based on T1-weighted MRI contrast should be evaluated.

In this paper, signal intensity changes of T1-weighted GE MRI according with the first-order concentration decay of paramagnetic nitroxyl contrast agents were calculated by assuming possible in vivo experimental conditions. Whether this surrogate decay rate calculated based on decreasing T1-weighted MRI contrast can be used as a fallback position was discussed to ensure in vivo redox mapping based on T1-weighted MRI, which was reported elsewhere.\textsuperscript{27–29} In addition, the suitability of conventional nitroxyl paramagnetic contrast agents, such as 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL) and 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-N-oxyl (CmP), as redox probes for MRI redox imaging based on T1-contrast was also discussed.
MATERIALS AND METHODS

Materials CmP and TEMPOL were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, U.S.A.). 4-Oxo-2,6,6-tetramethylpiperidine-1-{15}N-oxyl (15N-TPN) was purchased from CDN Isotopes Inc. (Quebec, Canada).

MRI Measurement The phantom was composed of seven tubes (4.7 mm i.d.) containing 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0 mM aqueous solution of a nitroxyl contrast agent, i.e., CmP, TEMPOL, or 15N-TPN. The shape of the phantom is shown in Fig. 1A. MRI measurement was performed at 4.7 T controlled with Paravision® 3.0.1 (Bruker BioSpin MRI GmbH, Ettlingen, Germany). SPGR (also referred to as gradient echo fast imaging, GEFI) (TR=75 ms, TE=3 ms, FA=45°, NEX=16) was employed to observe the T1 effect, where NEX is the number of excitations and see Table 1 for other abbreviations. The scan time for an image set (which included 2 slices) by the SPGR sequence was 120 s. Other image parameters are as follows: image resolution was 256×256, field of view (FOV) was 3.2×3.2 cm, slice thickness was 2.0 mm. Sample temperature was monitored and kept at 37 °C using a water circulating system. ΔM%, which is the enhancement of image intensity from the baseline, was calculated using the intensity of the center tube (water) as the baseline:

\[ \Delta M\% = \frac{\text{pixel intensity} \times (\text{average intensity in the center tube}) - 100 - 100}{100} \]  

(1)

MRI data were analyzed using the ImageJ software package (a public domain Java image-processing program inspired by NIH Image that can be extended by plug-ins, http://rsb.info.nih.gov/ij/).

Simulation SPGR image intensity \( M_i \) in a tissue at time \( t \) (min) after injection of a nitroxyl contrast agent was calculated by an equation:

\[ M_i = M_0 \times \left[ 1 - \frac{\Delta M\%}{100} \mathrm{exp}\left(-\frac{TR \times \Delta t}{T_1^*}\right) \right] \times \left[ 1 - \frac{\Delta M\%}{100} \mathrm{exp}\left(-\frac{TE}{T_2^*}\right) \right] \times \left[ \sin(FA) \left[ 1 - \cos(FA) \mathrm{exp}\left(-\frac{TR \times \Delta t}{T_1^*}\right) \right] \right] \]  

(2)

where \( M_0 \) is proton density and FA is the flip angle. \( T_1^* \), which is the \( T_1 \) value obtained in the tissue at a particular time \( t \), changes depending on the concentration of the nitroxyl contrast agent \( (C_t) \) in the tissue. \( T_2^* \) was assumed to be constant before and after injection of the nitroxyl contrast agent in this simulation. The concentration of nitroxyl contrast agent applied in this simulation was low enough to eliminate the effect to \( T_2^* \).

\[ T_1 = \frac{1}{R_1} \]  

(3)

\[ R_1 = \frac{1}{T_1} + r_i \times C_t \]  

(4)

where \( R_1 \) is the \( T_1 \) relaxation rate at time \( t \), \( T_1 \) is the initial \( T_1 \) baseline (intrinsic \( T_1 \) of tissue itself), and \( r_i \) is the relaxivity of the nitroxyl contrast agent.

Table 1. Variables Used in the Simulation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Unit</th>
<th>Values input</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_t )</td>
<td>Concentration of the contrast agent at time ( t )</td>
<td>mm</td>
<td>Calculated by Eq. 5</td>
</tr>
<tr>
<td>( C_{max} )</td>
<td>Concentration of the contrast agent at ( t=0 )</td>
<td>mm</td>
<td>0.25, 0.5, 1.0, 1.5, 2.0</td>
</tr>
<tr>
<td>( \Delta M% )</td>
<td>Percentage enhancement of image intensity at time ( t )</td>
<td>%</td>
<td>Calculated by Eq. 6</td>
</tr>
<tr>
<td>Error%</td>
<td>Percentage difference of ( k_{\text{fin}} ) from ( k_{\text{ini}} )</td>
<td>%</td>
<td>Calculated by Eq. 7</td>
</tr>
<tr>
<td>FA</td>
<td>Flip angle</td>
<td>degree</td>
<td>10, 15, 30, 45, 60, 90</td>
</tr>
<tr>
<td>( k_{\text{fin}} )</td>
<td>Decay rate calculated using ( \Delta M% )</td>
<td>min(^{-1} )</td>
<td>0.02—1.50</td>
</tr>
<tr>
<td>( k_{\text{ini}} )</td>
<td>Original decay rate</td>
<td>min(^{-1} )</td>
<td>1000</td>
</tr>
<tr>
<td>( M_0 )</td>
<td>Proton density</td>
<td>a.u.</td>
<td>Calculated by Eq. 2 when ( T_1=0 )</td>
</tr>
<tr>
<td>( M_i )</td>
<td>SPGR image intensity of tissue (baseline intensity)</td>
<td>a.u.</td>
<td>Calculated by Eq. 2</td>
</tr>
<tr>
<td>( t )</td>
<td>Time after injection of contrast agent</td>
<td>min</td>
<td>Incremented from 0 to 30</td>
</tr>
<tr>
<td>( T_1 )</td>
<td>Intrinsic ( T_1 ) of tissue</td>
<td>ms</td>
<td>250, 500, 750, 1000, 2000, 2350</td>
</tr>
<tr>
<td>( T_1 )</td>
<td>( T_1 ) at time ( t )</td>
<td>ms</td>
<td>Calculated by Eq. 3</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
<td>ms</td>
<td>3</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
<td>ms</td>
<td>75, 80, 85, 90, 95, 100</td>
</tr>
<tr>
<td>( R_1 )</td>
<td>( T_1 ) relaxation rate (( R_1 )) at time ( t )</td>
<td>ms(^{-1} )</td>
<td>Calculated by Eq. 4</td>
</tr>
<tr>
<td>( r_i )</td>
<td>( T_1 ) relaxivity of the nitroxyl contrast agent</td>
<td>mm(^{-1} )</td>
<td>0.10, 0.13, 0.15, 0.2, 0.3, 0.5</td>
</tr>
</tbody>
</table>

Underlined numbers were used as default values, which are the parameters used in the phantom experiment and estimated relaxivity for a nitroxyl contrast agent. \( T_1^* \) was 50 ms.

Fig. 1. Nitroxyl Enhanced \( T_1^* \)-Weighted SPGR-Based MRI Signals
(A) A phantom consisted of 7 glass tubes (4.7 mm i.d.) containing different concentrations of a nitroxyl contrast agent solution in water. Numbers in the figure are concentrations of the nitroxyl contrast agent in mM. Three phantoms were prepared for three different nitroxyl contrast agents, i.e., CmP, TEMPOL, and 15N-TPN. (B) \( T_1 \)-weighted MR image of the phantom containing CmP, for example. Scan conditions were as follows: TR=75 ms, TE=3 ms, FA=45°, NEX=16, slice thickness 2 mm, FOV 32×32 mm, resolution 256×256. (C) \( \Delta M\% \) values from the MR image were plotted against the concentration of several nitroxyl contrast agents. The dotted line indicates the simulated line, shown in Fig. 2. The experiment was performed under temperature control at 35 °C using a hot water cycling pad wrapped around the resonator.
ity of the nitroxyl contrast agent. $C_0$ (mm), which is the concentration of the nitroxyl contrast agent in the tissue at a particular time $t$, was calculated by assuming a first-order decay equation:

$$C_t = C_{max} \times \exp(-k_{true} \times t)$$

where $k_{true}$ is the given decay rate. Logarithmic values of the intensity enhancement (difference from the baseline), i.e.,

$$\Delta M^\% = (M/M_0 - 1) \times 100$$

were plotted against time $t$. $M_i$ is the intrinsic signal intensity of tissue calculated as $C_i=0$. The decay constant $k_{MRI}$ is obtained from the slopes of plotted $\Delta M^\%$, using least squares fit through origin. All calculations and plots were carried out using Microsoft Excel XP. The variables used in this simulation are summarized in Table 1.

RESULTS

A phantom study showed the relation between $T_1$-contrast enhancement ($\Delta M^\%$) and the concentration of a nitroxyl contrast agent experimentally. MR image intensity shows the concentration-dependent difference of solutions in tubes (Fig. 1B). For phantom experiments, the average intensity of the center tube (water) was used as the baseline to calculate $\Delta M^\%$ for each tube. Therefore, the fluctuations of $\Delta M^\%$ values were large in such baseline correction. Slopes between experimentally obtained $\Delta M^\%$ and the concentrations of nitroxyl contrast agents, however, showed similar slopes around the simulated one (dotted line in Fig. 1C). The simulation was performed as below.

Figure 2 shows the calculated MRI intensity change ($\Delta M^\%$) depending on the concentration of a nitroxyl contrast agent in water. $T_1$ relaxivity for a nitroxyl contrast agent at $37^\circ C$ was experimentally estimated as $r_1=0.13 \text{ mm}^{-1} \text{s}^{-1}$ from $T_1$, mapping of the same phantoms used in previous experiments. $T_1$ of the water used in this simulation, i.e., $T_1w$, was estimated as 2350 ms. The relation between the concentration of the paramagnetic contrast agent and $T_1$-weighted signal enhancement was not actually linear in a wide concentration window (0—10 mM) (Fig. 2A), while relatively good linearity was obtained in a lower concentration window (0—2 mM) (Fig. 2B). The profile shape of the $\Delta M^\%$ concentration curve changed depending on relaxivity $r_1$. The linear region became shorter with larger $r_1$ (data not shown).

Time courses of simulated MRI contrasts, $\Delta M^\%$, were calculated using several decay rates as input values, i.e., $k_{true}$ from 0.02 to 1.50 min$^{-1}$. A plot of $\ln(\Delta M^\%)$ versus time $t$ gave an almost linear decay, and then the decay constant of $\Delta M^\%$, i.e., $k_{MRI}$, was estimated from the slope of an identical time window from $t=0$ to $t=5$ min. The $k_{MRI}$ showed a similar but slightly smaller value than the given $k_{true}$. The error between $k_{MRI}$ and $k_{true}$ (percentage difference of $k_{MRI}$ from $k_{true}$), Error%, was estimated as below:

$$\text{Error}\% = \left(1 - \frac{k_{MRI}}{k_{true}}\right) \times 100$$

The relationship between Error% and $k_{true}$ was observed under several conditions and compared.

Figure 3A indicates the relationship between Error% and the given $k_{true}$ when the given $C_{max}$ was changed. Other parameters used in the calculation were fixed as follows: TR = 75 ms, TE = 3 ms, FA = 45°, and $T_1$ = 2350 ms. Error% became larger as the given $k_{true}$ became smaller. When the given $C_{max}$ became larger, the Error% became larger and sensitivity increased in the lower $k_{true}$ region. Error% was, however, under 5% for all cases simulated here.

Similarly, Fig. 3B indicates the relationship between Error% and the given $k_{true}$ values obtained using several $T_1$ values as inputs. Other parameters used in the calculation were fixed as $C_{max}=1.5$ mm, $TR=75$ ms, TE = 3 ms, and FA = 45°. Error% became larger when the given $k_{true}$ became smaller. When the given $C_{max}$ became larger, the Error% became larger and sensitivity increased in the lower $k_{true}$ region. Error% was, however, under 5% for all cases tested here.

Figure 3C indicates the relationship between Error% and the given $k_{true}$ values obtained using several FA values as inputs. Other parameters used in the calculation were fixed as follows: $C_{max}=1.5$ mm, $T_1=2350$ ms, TE = 3 ms, and FA = 45°. The effect of TR on Error% was small and Error% was under 5% for all cases tested here.

Figure 3D indicates the relationship between Error% and the given $k_{true}$ values obtained using several $T_1$ values as inputs. Other parameters used in the calculation were fixed as follows: $C_{max}=1.5$ mm, $T_1=2350$ ms, TE = 75 ms, and FA = 45°. Error% very slightly changed when inputs of FA changed; however, the effect of FA was small. Error% was under 5% for all cases tested here.

Figure 3E indicates the relationship between Error% and the given $k_{true}$ values obtained using several $T_2^*$ values as inputs. Other parameters used in the calculation were fixed as follows: $C_{max}=1.5$ mm, $T_1=2350$ ms, TE = 3 ms, and FA = 45°. Error% very slightly changed when inputs of $T_2^*$ changed; however, the effect of $T_2^*$ was small. Error% was under 5% for all cases tested here.
The potential of nitroxyl radicals and/or hydroxylamines as a redox-sensitive contrast agent for MRI has been proposed in earlier studies.\textsuperscript{1,13,32} Recent high-power magnet MRI instrumentation, such as 4.7 T, with a T$_1$-weighted SPGR pulse sequence, has enabled us to examine T$_1$ enhancement sensitively, for example, T$_1$ enhancement under hyperbaric oxygenation in mice.\textsuperscript{31} Scan parameters used in the phantom experiment in this paper were employed according to the parameters used in our previous paper.\textsuperscript{33} Suitable values of these parameters were estimated by previous simulation, and then decided through practical experiments to detect T$_1$ enhancement by oxygen \textit{in vivo}. When nitroxyl contrast agents were applied to the same T$_1$-weighted pulse sequence scan,\textsuperscript{27—29} a more pronounced contrast difference (ΔM%) was expected to be obtained.

The reciprocal of T$_1$, \textit{i.e.}, relaxation rate R$_1$ = 1/T$_1$, has a linear relation to the concentration of the contrast agent. The enhancement magnitudes of T$_1$-weighted signal intensity by the contrast agent do not have a linear relation to the concentration of the contrast agent. An approximately linear relation can be obtained for the lower concentration region, when relativity $r_1$ was small ($r_1$ was 0.13 mm$^{-1}$ s$^{-1}$ in this simulation). Although this is not exactly linear, quantification in such a concentration region is possible when an identical solvent, \textit{i.e.}, identical T$_1$, is considered for all experiments. When animal experiments are considered, however, quantification is difficult. Signal intensity $M_s$ can be calculated by Eq. 2, in which $M_s$ is a function of T$_s$, T$_1$, is composed of intrinsic tissue T$_1$, \textit{i.e.}, T$_1$, and induced T$_1$ by a contrast agent, \textit{i.e.}, 1/($r_1 \times C_t$). T$_1$ was composed of the addition of $R_k$ (reciprocal of T$_1$); therefore, this addition causes a difference in the slope of ln(ΔM%), \textit{i.e.}, $k_{MRI}$, at each time point and shows the error from $k_{true}$.

It can be predicted that the variables in Eq. 2 will cause errors between $k_{true}$ and $k_{MRI}$. In fact, Error% was larger with slower $k_{true}$, larger $C_{max}$, shorter T$_1$, longer TR, smaller FA, and/or larger $r_1$, TE and intrinsic tissue T$_s$, \textit{i.e.}, T$_s$, affected the values of $M_t$, similar to ΔM%, but not the decay rate of ln(ΔM%). Effects of scan parameters, \textit{i.e.}, TR and FA, on Error% were sufficiently small, and Error% was always below 5% in this simulation when default values were used as other inputs. The slow decay rate should be estimated carefully, especially in tissue with shorter T$_1$. In addition, approximation of the slower decay rate may become inaccurate when a nitroxyl contrast agent with large $r_1$ is used. Effects of experimental parameters, \textit{i.e.}, $C_{max}$, can be adjusted by choosing the experimental dose or by choosing a time window for estimating the decay rate in data manipulation.

Under identical experimental conditions, \textit{i.e.}, identical dose of a contrast agent and fixed scan parameters, $k_{MRI}$ values will be slightly affected only by T$_s$. Error% can be less than 4% for $k_{true}$ faster than 0.02 min$^{-1}$, when T$_1$ is 750 ms, TR is 75 ms, and FA is 45°. Error% can be smaller for faster $k_{true}$, and less than 2% when $k_{true}$ is larger than 0.3 min$^{-1}$. Effect of T$_s$ on Error% was sufficiently small when given T$_s$ was longer than 500 ms; however, Error% became slightly higher when the given T$_s$ was 250 ms. T$_s$ values of approximately 790 and 1000 ms for normal muscle and SCC tumor tissues, while approximately 250 ms for fat tissue, can be estimated from our previous work.\textsuperscript{32} Several tissues, such as the liver and brain (white matter), may show 500—600 ms T$_1$; therefore, the estimation of tissue redox status based on the time course of nitroxyl-caused T$_1$ enhancement can be realized in practical \textit{in vivo} experiments targeting muscle, brain, or other soft tissues, except when tissues have shorter T$_1$, such as fat. Comparison of $k_{MRI}$ among different tissues needs careful consideration of the differences achieved by different tissue-specific T$_1$, especially for tissues with short
T₁. Preliminary T₁ mapping of the experiment is recommended.

When a general in vivo experimental dose (<1.5 μmol/g body weight (b.w.) by bolus injection) of a nitroxyl contrast agent was administered via intravenous (i.v.) injection to a mouse, blood concentration may immediately increase, and then start to decay through diffusion, reduction, and excretion; therefore, initial high blood concentration may underestimate kMRI. When 1.5 μmol/g b.w. of TEMPOL or CmP was injected by bolus i.v. administration, the blood concentration of the nitroxyl contrast agent is expected to be below 1.5 mM, 0.8 and 2.5 min after the injection, respectively. At the same time, the tissue concentration of either TEMPOL or CmP is predicted to be below 0.5 mM. Since the quite fast in vivo decay rates of TEMPOL may be faster than 1.0 min⁻¹, errors between ktrue and kMRI might remain below 2.8%, even if Cmax is 6 mM. The blood concentration of 6 mM of TEMPOL can reach 0.4 min after i.v. administration. The kMRI should therefore be estimated from data after those time points. This suggests that conventional nitroxyl paramagnetic contrast agents, such as TEMPOL and CmP, can be good candidates for redox probes for MR redox imaging based on T₁ contrast. In contrast, membrane-impermeable nitroxyl contrast agents, such as carboxy-PROXYL, may maintain a high blood concentration for a relatively long time, are not suitable MR redox probes.

Changing T₁i and T₂* before and after injection of the nitroxyl contrast agent should be considered carefully, because these changes can give relatively large errors in kMRI values. The difference between kMRI and ktrue will become larger, over 10%, when ca. 0.1% of T₁ shortening is assumed as a result of the pharmacodynamic effect of the contrast agent. The kMRI is increased when T₂* is shortened after injection of a contrast agent; however, the error of kMRI can remain in the range of ±5% when T₂* shortening after injection is less than 5%. In addition, the effect on T₂* shortening of the nitroxyl contrast agent is sufficiently small under the concentration used in general in vivo experiments. The difference of kMRI from ktrue becomes larger when the original T₁i is shorter and when the same R₂ change occurs by injection of the contrast agent. Similarly, the difference of kMRI from ktrue becomes larger when the original T₂* is shorter and when the same R₂ change occurs. A physiological change of T₁i and T₂* can be expected by a relatively large change of blood flow, blood pressure, etc.; therefore, careful control of anesthetic conditions, body temperature, and breathing rate is necessary for in vivo animal experiments. The kMRI values are complicated even under identical experimental conditions, when such T₁i and T₂* changes are observed dose dependently. Fortunately, physiological T₁i and T₂* changes caused by injection of the nitroxyl contrast agent itself are expected to be sufficiently small using a suitable low dose, i.e., under general in vivo experimental conditions.

Nitroxyl contrast agents having a single free radical on the molecule showed relatively smaller relaxivity (0.13 mM⁻¹ s⁻¹ at 4.7 T at 37 °C); however, such low relaxivity achieves good linearity between its T₁ contrasts and the concentrations of nitroxyl contrast agents, especially at lower concentrations. Good linearity (R²≥0.9995) was obtained at less than 2 mM for the nitroxyl contrast agent by simulation. This linearity improved in a lower concentration region (R²≥0.9997 at less than 1.5 mM), while linearity fell for a Gd³⁺-based contrast agent, which has a relaxivity of around 4 mM⁻¹ s⁻¹ at 4.7 T, and the acceptable linear region (R²≥0.999) becomes much shorter (<0.125 mM approximately). A contrast agent with lower relaxivity can give better quantification ability, while lower relaxivity will result in low SNR. In contrast, higher relaxivity can give good SNR; however, higher relaxivity gave lower linearity of T₁ contrast vs. concentration of the contrast agent. A tri-radical nitroxyl contrast agent, which has relaxivity around 5 mM⁻¹ s⁻¹, may be compoundable.

For exact quantification, the T₁ relaxation time (T₁ relaxation rate) is a quantitative value; however, the generally used spin echo-based quantitative T₁ measurement has too long an acquisition time to estimate the pharmacokinetics of nitroxyl contrast agents. Recently, a super-fast T₁ measurement method has been developed using an EPI-based sequence. Using the time course of T₁ values instead of T₁-weighted contrasts, an estimation of the true decay rate ktrue may be available in the future.

Currently, the T₁-weighted contrast-based pseudo decay rate, i.e., kMRI, is a simplified tool for redox imaging when the experiment is performed under general biological conditions. Although the absolute concentration of the contrast agent is difficult to estimate from T₁-weighted contrast, the approximate decay constant (kMRI) of the nitroxyl contrast agent estimated from the time course of T₁-weighted contrast can be an alternative index of the redox status. Selecting a suitable nitroxyl contrast agent, using suitable scan conditions, and achieving scout T₁ mapping of the identical slice, the decay rate of T₁-weighted MRI can be utilized as the primary index of redox information in a biological sample.

CONCLUSION

The time course of decreasing T₁ contrast according with the first-order decay (ktrue) of the nitroxyl contrast agent was simulated, and the decay rates of MR T₁ contrast (kMRI) were obtained for several parameter settings. The kMRI gave a slightly smaller value than the given ktrue. Although kMRI was affected by the initial (maximum) concentration of the paramagnetic nitroxyl contrast agent, intrinsic tissue T₁, relaxivity of the probe, and scan parameters (TR and FA), the differences between kMRI and ktrue were sufficiently small when parameters in practical ranges were used. Under suitable experimental conditions, i.e., a practical/low dose of membrane-permeable nitroxyl contrast agents, such as TEMPOL and CmP, and a suitable T₁-weighted pulse sequence, kMRI can be an alternative index of the redox status. In vivo redox mapping based on T₁-weighted MRI can be an easy tool for redox imaging when the experiment is performed under general biological conditions.

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REFERENCES

1) Utsumi H., Yasukawa K., Soeda T., Yamada K., Shigemi R., Yao T.,


