Estimation of the Onset Time of Cerebral Ischemia Using $T_{1p}$ and $T_2$ MRI in Rats

Kimmo T. Jokivarsi, PhD; Yrjö Hiltunen, PhD; Heidi Gröhn, PhD; Pasi Tuunanen, PhD; Olli H.J. Gröhn, MD, PhD; Risto A. Kauppinen, MD, PhD

Background and Purpose—Time of ischemia onset is the most critical factor for patient selection for available drug treatment strategies. The purpose of this study was to evaluate the abilities of the absolute longitudinal rotating frame ($T_{1p}$) and transverse ($T_2$) MR relaxation times to estimate the onset time of ischemia in rats.

Methods—Permanent middle cerebral artery occlusion in rats was used to induce focal cerebral ischemia and animals were imaged with multiparametric MRI at several time points up to 7 hours posts ischemia. Ischemic parenchyma was defined as tissue with apparent diffusion coefficient of water <70% from that in the contralateral nonischemic brain.

Results—The difference in the absolute $T_{1p}$ and $T_2$ between ischemic and contralateral nonischemic striatum increased linearly within the first 6 hours of middle cerebral artery occlusion. The slopes for $T_{1p}$ and $T_2$ fits for both tissue types were similar; however, the time offsets were significantly longer for both MR parameters in the cortex than in the striatum.

Conclusions—$T_{1p}$ and $T_2$ MRI provide estimates for the onset time of cerebral ischemia requiring regional calibration curves from ischemic brain. Assuming that patients with suspected ischemic stroke are scanned by MRI within this timeframe, these MRI techniques may constitute unbiased tools for stroke onset time evaluation potentially aiding the decision-making for drug treatment strategies. (Stroke. 2010;41:2335-2340.)

Key Words: acute stroke ■ animal models ■ brain imaging ■ brain ischemia ■ MRI

Collapse of adenosine 5'-triphosphate due to acute ischemia is associated with subtle changes in both ionic and water homeostasis in the brain. Altered homeostasis in the brain is probed by MR techniques to follow ionic and water homeostasis in the brain. Altered homeostasis in stroke.

Several neuroprotective drugs have shown an effect in adverse effects.4,5 Similarly, other treatment methods such as hypothermia provide a beneficial outcome when applied within a time window from stroke onset.7

Several neuroprotective drugs have shown an effect in preclinical settings only when administered within a narrow time window from stroke onset.7

It is well established by electrophysiological and tissue ion analysis techniques that brain sodium increases in a linear fashion after ischemia.8 Based on this fact, it has been shown that $^{23}$Na MRI signal intensity increases as a function of ischemia duration; thus, $^{23}$Na signal has been termed a “ticking clock of stroke.”9,10 However, only few clinical centers have MR scanners capable of $^{23}$Na MRI. The currently exploited MRI techniques for stroke diagnosis, the diffusion and perfusion MRI, are excellent for detecting acute ischemia; however, their value in evaluating ischemia duration from one time point measurement is still debated.11 Recently, using absolute $T_2$ relaxation time MRI in a stroke patient population, it was reported that the difference between ischemic and nonischemic contralateral $T_2$ ($\Delta T_2$) increases quadratically between 1 and 6 hours after symptom onset.12 In agreement with Siemonsen et al.,12 combined information from diffusion and fluid-attenuated inversion recovery imaging has been suggested as a means to estimate the time of the ischemia onset.13,14 The increase in $T_2$ poststroke has been ascribed chiefly to an increase in tissue water.12 It should be noted, however,
that other factors influence $T_2$, including the blood oxygenation level-dependent effect. Furthermore, brain $T_2$ is dependent on the external magnetic field requiring calibration data for each field used.

$T_1$/H9267 represents the longitudinal rotating frame relaxation time. $T_1$/H9267 signal is sensitive to changes in interaction between water and macromolecules, including dipolar interactions and chemical exchange. Ischemia causes changes in the tissue water environments, pH, and temperature leading to substantial changes in $T_1$/H9267, which make it a sensitive and early index of irreversible ischemia resulting from middle cerebral artery occlusion (MCAO). $T_1$/H9267 MRI has been acquired from human brain using clinical scanners. The aim of the current study was to examine the ability of absolute $T_1$/H9267 and $T_2$ to indicate the onset time of ischemia in rat models of permanent stroke.

**Materials and Methods**

**Animal Model**

Male Wistar rats ($n=12$, weight 200 to 300 g; Animal Resource Facility, University of Eastern Finland, Kuopio, Finland) were exposed to permanent MCAO according to Longa et al.21 The occluding thread was left in place for the duration of the MRI scanning and the animals were euthanized thereafter. One animal died during the imaging and was excluded from the data set. Stroke lesion was confirmed by $T_2$ MRI 5 to 7 hours post-MCAO.

All operations and scanning were done under isoflurane anesthesia with a constant flow of 70/30 N2O through a face mask. The core temperature was monitored online and was maintained close to 37°C by circulating warm water in a heating pad placed under the torso. Breathing rate was also monitored throughout the MRI study (SA Instruments Inc). Arterial blood gases and pH were analyzed immediately before MR scanning (i-Stat Co, East Windsor, NJ). All animal procedures were approved by the Animal Care and Use Committee of the University of Eastern Finland and conducted in accordance with the guidelines set by the European Community Council Directives 86/609/EEC.

**Magnetic Resonance Imaging**

The MRI experiments were performed in a horizontal 4.7-T Magnex Scientific Inc (Yarmton, UK) magnet interfaced to a Varian Inova console (Palo Alto, Calif). MRIs were acquired at several time points with 30- ($N=7$) or 60- ($N=4$) minute intervals during MCAO. A volume coil transmit/quadrature half-volume receive setup was used (Rapid Biomedical GmbH, Rimpar, Germany).

Fast spin-echo readout data (64×128 pixels, echo spacing 10 ms, field of view 2.56×2.56 cm$^2$) was used for $T_1$/H9267 and $T_2$ MRI. The on-resonance spin-lock $T_1$/H9267 MRI was acquired with a continuous wave-$T_1$/H9267 approach. A contrast formation block of AHP-SL-AHP (AHP=adiabatic half passage, SL=spin-lock) segment was added before readout. Adiabatic spin-lock pulses ranging from 8 to 64 ms were used ($B_{1,SL}$ 0.4 G, time to repetition of 2.5 seconds and time to echo [TE] of 6 ms). $T_2$ images were computed from the multi-TE data sets (5 TEs) using the fast spin-echo sequence with a preparation block consisting of adiabatic pulses (AHP-TE/4-AFP-TE/2-AFP-TE/4-reverse AHP, AFP=adiabatic full passage). Time to repetition and TE within the fast spin-echo readout were set similar to those used with the SL acquisitions.

The trace of diffusion tensor ($D_{av} = \frac{1}{3} \text{Trace}[D]$) image was used to localize the acutely ischemic tissue. $D_{av}$ was quantified using a spin-echo MRI sequence incorporating 4 bipolar gradients along each axis with 4 $b$-values ranging from 0 to 1370 s/mm$^2$ (time to repetition 1.5 seconds, TE 55 ms). $B_1$ map was calculated for the homogeneity control of the radiofrequency field. A cosine function was fitted to signal intensity oscillation that was caused by a variable length square preparation pulse and a crusher gradient in front of a fast low-angle shot pulse.

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**Figure 1.** A, Localized ROIs in striatal (Str) and cortical (Cor) locations in both ipsilateral (Ipsi) and contralateral (Cont) sides. B, Mean $\Delta T_{1p}$ and $\Delta T_2$ as a function of time from the occlusion in permanent MCAO data for each animal in Str and Cor locations and the corresponding linear trend lines. $\Delta T_{1p}$ trendline for striatum was forced through 0.
sequence (time to repetition 4.5 ms, TE 2.2 ms) as previously described.24

Data Analysis

Values for each MRI variable were calculated as the mean from small regions of interest (ROI; approximately 3 mm²) positioned at the core of the ischemic lesions in the striatum and basal cortex (Figure 1A). The location for the ROI was guided visually based on the changes in $D_\text{av}$ at approximately 1 hour from MCAO ($D_\text{av}$ decrease by >30% in striatum and in basal cortex). Corresponding contralateral ROIs were chosen as reference. $\Delta T_{1p}$ and $\Delta T_2$ were calculated as the difference between the mean ipsilateral and contralateral values using an in-house-written Aedes software (http://aedes.uku.fi) under Matlab routine (MathWorks, Natick, Mass.).

Linear least squares fit was used to calculate the correlation coefficients ($R$). The 95% CI for the mean was calculated as follows:

$$\text{mean} \pm t_{\alpha/2} \times \text{SEM}$$

where $t_{\alpha/2}$ is Student $t$ value for the 95% interval (N = 11) and SEM is the standard error of mean. All values are shown as mean±SD.

Results

$D_\text{av}$ decreased to 58%±9% in striatum and 56%±21% in basal cortex (values not significantly different) by 45 minutes of permanent MCAO and it stayed at this low level for the observation period of up to 7 hours. During MRI, the core temperature was 37.0±1.0°C and the breathing rate at 59±12 minutes⁻¹. Blood pH, $pO_2$, and $pCO_2$ were 7.31±0.05, 99±22 mm Hg, and 61±9 mm Hg, respectively, briefly before MRA sessions began.

The time courses for $\Delta T_{1p}$ from the striatum showed excellent correlations with the time from MCAO ($t_{\text{MCAO}}$; Figure 1B). A linear fit for the $\Delta T_{1p}$ data showed a correlation of

$$t_{\text{MCAO}} = (15.9±0.8) * \Delta T_{1p} + (11.4±10.7)$$

where $R$=0.897 ($P$<0.001). The fit was simplified to go through 0

$$t_{\text{MCAO}} = (16.7±0.4) * \Delta T_{1p}$$

where $R$=0.895 ($P$<0.001, not significant from the fit [1]). A linear fit for the $\Delta T_2$ data gave

$$t_{\text{MCAO}} = (21.9±1.0) * \Delta T_2 + (92.7±6.4)$$

where $R$=0.915 ($P$<0.001). Time-wise breakdown of $\Delta T_{1p}$ and $\Delta T_2$ values in absolute and relative terms together with the measured and estimated MCAO onset times and the 95% CIs are given in the Table.

Table. Changes in Striatal $T_{1p}$ ($\Delta T_{1p}$) and $T_2$ ($\Delta T_2$), Actual ($\Delta t$) and Estimated ($\Delta t_{\text{est}}$) Mean Times (±SD) From the Onset of Permanent MCAO, and the 95% CIs for Estimated Times Measured Within the Time Windows From the MCAO*

<table>
<thead>
<tr>
<th>Time From MCAO, Minutes</th>
<th>$\Delta T_{1p}$, ms</th>
<th>$\Delta T_{1p}$, %</th>
<th>$\Delta t$, Minutes</th>
<th>$\Delta t_{\text{est}}$, Minutes</th>
<th>95% CI, Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–60 (N=10)</td>
<td>3.1±1.7</td>
<td>4±2</td>
<td>41±6</td>
<td>52±29</td>
<td>32–70</td>
</tr>
<tr>
<td>60–120 (N=11)</td>
<td>6.4±2.6</td>
<td>9±4</td>
<td>93±17</td>
<td>107±43</td>
<td>78–136</td>
</tr>
<tr>
<td>120–180 (N=11)</td>
<td>9.5±2.5</td>
<td>13±3</td>
<td>154±15</td>
<td>158±41</td>
<td>131–186</td>
</tr>
<tr>
<td>180–240 (N=11)</td>
<td>12.7±3.2</td>
<td>17±4</td>
<td>213±17</td>
<td>219±53</td>
<td>177–248</td>
</tr>
<tr>
<td>240–400 (N=10)</td>
<td>17.1±3.0</td>
<td>23±4</td>
<td>308±43</td>
<td>283±49</td>
<td>254–319</td>
</tr>
</tbody>
</table>

*The estimated time ($\Delta t_{\text{est}}$) is calculated using Equations (2) and (3) for $\Delta T_{1p}$ and $\Delta T_2$, respectively. N indicates no. of animals constituting the data.

Discussion

The results demonstrate that both $\Delta T_{1p}$ and $\Delta T_2$ change in linear fashions after MCAO and they provide estimates for the onset (or duration) of ischemia in the striatum and basal cortex in the animal model that is in MRI terms close to human stroke without reperfusion. Although the slopes for both $\Delta T_{1p}$ and $\Delta T_2$ fits are similar in both tissue types, the time offsets differ significantly. Striatum forms the core of...
ischemia due to blood supply by end arterioles, whereas in the
basal cortex, the blood supply is provided by middle cerebral
artery and the circle of Willis. Variation in anatomy of blood
supply, together with other tissue type specific factors,
apparently results in differing time courses of the MR
relaxation time changes in striatum and cortex during the
course of ischemia.

$T_1$ MRI signal is influenced by total tissue water
content, as is $T_2$, but $T_1$ is evidently much less affected
by the blood oxygenation level-dependent effect than $T_2$.25 It
has been shown that tissue water content increases after
ischemia depending on the degree of vascular occlusion.26 In
the permanent MCAO of rat, total water content has
been shown to remain unchanged for the first 2 hours of
ischemia.27,28 In fact, delayed increase in tissue water
content is the key reason behind the low sensitivity of CT to
to acute stroke.27 Thus, there is evidence to show that total
tissue water content does not increase significantly within
the first hour of ischemia and, therefore, cannot explain
$T_1$ or $T_2$ MR signal increase.29 It should be stressed that
MRI signal, weighted for either diffusion or relaxation, is
influenced by several other physicochemical factors than
total water concentration. These factors, including distribu-
tion of tissue water between extracellular and intracel-
lar space, breakdown of cytoskeleton, pH, chemical
exchange between amide protons and bulk water, and
temperature, undergo much earlier alterations than what is
determined for total water content. Isochronous chemical
exchange is a relaxation mechanism for $T_1$ and it is likely
that in the ischemic brain physicochemical conditions will
slow down chemical exchange affecting $T_1$.30 We believe
that these physicochemical factors influence $T_1$ MRI
signal in the early moments of ischemia before the increase
in total tissue water becomes the key contributor to both
$T_1$ and $T_2$ MRI signals.

The current $T_2$ results from the animal stroke model
support the clinical observations that absolute $T_2$ of brain
tissue provides information about the duration of ische-
mia.12 However, during the early minutes of ischemia,
single Hahn echo $T_2$ MRI shows $T_2$ shortening observed
both at 4.7 T and 9.4 T due to the negative blood oxygenation
level-dependent effect.18,31 Shortening of $T_2$ has also been reported acutely in patients imaged at 1.5 T.32 The presence of such $T_2$ shortening results in a
situation in which there is a time point when $T_2$ in the
stroke tissue shows no difference to contralateral nonis-
chemic tissue. In our data set, the $\Delta T_2$ zero crossover time
greatly varies between striatum and cortex. This is likely to
cause error in time estimation from a single time point $T_2$
MRI in the early moments of stroke. We consider this a
potential weakness for $T_2$ MRI in clinical settings where
estimation from symptom onset is often complicated.12
Further issues include the fact that $T_2$ measurement in vivo
is not trivial due to technical reasons and the $T_2$ value is
influenced both by the interval between refocusing pulses
and stimulated echoes potentially generated within multi-
cho sequences. Because of these factors, $T_2$ should be
 calibrated for clinical settings for each pulse sequence and
perhaps even for each scanner. These factors are not an
issue for $T_1$, and $T_1$ measurement is expected to be more
consistent provided that $B_1$ of the SL field is uniform.
Nevertheless, it is advisable to presently use absolute $T_2$
data estimation of stroke duration, because the majority of
clinical MRI scanners are equipped with techniques to
quantify $T_2$ within a few minutes. Sensitivity of $T_2$ to
to blood oxygenation level-dependent can be modulated by
pulse sequence choice. For instance, a short interpulse
Carr-Purcell-Meiboom-Gill sequence has much reduced
sensitivity to blood oxygenation level-dependent, as shown
experimentally at 4.7 T.33

Figure 2. A scheme for estimation of ischemia
time. $D_{sv}$ image is used to identify the ischemic
tissue and an ROI (white circle) is selected. The
difference of the means of the homologous (ipsi-
ateral and contralateral ROIs in the $\Delta T_1$) image is then calculated. A multiplication from the
linear fit is used for the ischemia time estimation.
Here, $\Delta T_1$ image was acquired 76 minutes after
MCAO.
A drawback for applying $T_{1p}$ clinically is the energy absorption (specific absorption rate), which inevitably limits the SL field amplitude and therefore the MR contrast from acute stroke. Before the era of clinical diffusion MRI, Sipponen and coworkers demonstrated that $T_{1p}$ MRI is able to diagnose acute stroke in humans using a low-field MRI scanner. Specific absorption rate scales as second power of both magnetic and $B_1$ fields. However, even with higher $B_1$ fields that could be used to increase the contrast, there was no significant tissue heating involved after a $T_{1p}$ measurement of a flow-compromised tissue. We have recently used adiabatic radiofrequency waveforms for $T_{1p}$ MRI with reduced specific absorption rate. The adiabatic $T_{1p}$ makes it possible to obtain contrast in acutely ischemic brain tissue with 20% to 80% lower specific absorption rate at the given SL amplitude, which is a promising factor toward clinical implementation of $T_{1p}$ MRI.

A scheme is suggested for estimation of ischemia time (Figure 2) that will take <5 minutes to complete. The presence of ischemia is confirmed by diffusion MRI and an absolute $T_{1p}$ image is acquired (acquisition <2 minutes) from preselected ischemic brain. An ROI representing the ischemic tissue is selected from diffusion image (1 minute). Ischemia time will be obtained from the difference in absolute $T_{1p}$ values between the ROI and ROI in the contralateral brain using a calibration curve from data bank (1 minute). In cases in which contralateral ROI shows diffusion abnormality, slice average data from the contralateral brain may be used. The data presented apply to the rat brain regions of striatum and basal cortex and separate regional calibration curves for human brain must be established.

In conclusion, we have shown that absolute $T_{1p}$ and $T_2$ MRI provide estimates of stroke onset in the permanently ischemic brain parenchyma within 6 hours of insult. $T_{1p}$ MRI has the potential to outperform $T_2$ MRI for a single time point imaging to this end, because $T_{1p}$ change is positive and linear during the evolution of stroke. The current data encourage the implementation of $T_{1p}$ MRI into the clinical imaging protocol of acute stroke.

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Disclosures

None.

References

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**Abstract**

Estimation of the Onset Time of Cerebral Ischemia Using $T_1$ and $T_2$ MRI in Rats

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文章要旨：脳血虚発症時間の推定

$T_1$ および $T_2$ MRI を用いたラットにおける脳血虚発症時間の推定

脳虚血発症時間は、薬物治療戦略に適した患者を選択する際の最も重要な要素である。本研究の目的は、MRIにおける回転フレームの $T_1$ 緩和時間 ($T_{1R}$) および $T_2$ 緩和時間 ($T_{2R}$) の絶対値を用いて、ラットにおける虚血発症時間を推定できるか否かを検討することであった。

方法：ラットの大舗脳脹脛に恒久的な閉塞を作製し、局在性脳虚血を誘発した上で、虚血後5、7, と14時間において複数の時点でmultiparametric MRIによる撮像を行った。虚血のない対側の脳に比べて水の見かけの拡散係数が70％未満の組織を、虚血が生じた実質とみなした。

結果：大脳動脈閉塞後14時間のうちに、虚血を生じた線条体と虚血のない対側の線条体における $T_{1R}$ および $T_{2R}$ の絶対値の差は線状に増加した。組織の種類にかかわらず、$T_{1R}$ および $T_{2R}$ グラフの傾きに違いはみられなかったが、いずれのMRパラメータでも、時間差は線条体よりも皮質の方が有意に大きかった。

結論：$T_{1R}$ および $T_{2R}$ MRI によって脳虚血発症時間を推定することができるが、虚血を生じた脳の局所正規曲線が必要である。虚血性脳卒中が疑われる患者のMRIが上記時間内に実施されるとすれば、本法は脳卒中発症時間を短く評価する手段として、薬物治療戦略の決定に役立つと考えられる。

Stroke 2010; 41: 2335-2340

![Image](stroke5_4.indb_34)