Evolution of cerebral ischaemia induced by thromboembolism in rats detected by early sequential MR imaging

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Thromboembolic stroke appears to evolve in patients in a very complicated manner. The present study investigated the evolution of thromboembolic stroke in rats (n=9) using a 4.7-T MR imager. Under isoflurane anaesthesia, the rats received homologous blood clots into the right internal carotid artery. After thromboembolic stroke, lesion volume, which was defined and calculated, based on apparent diffusion coefficient maps, tended to increase gradually over the 6 h magnetic resonance imaging study. The largest percentage change in lesion volume was found at the early stage (40–100 min) of thromboembolism, and showed significant correlation with total percentage change in lesion volume (41.6 (SD 32.8%)) (r=0.77, P<0.05). In conclusion, marked enlargement or diminution of lesion volume may be observed at the early stage of thromboembolism. Thromboembolic stroke, which can be partly salvaged at the early stage, may likely evolve to a lesser extent thereafter.

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diffusion weightings. This raises the possibility that the extent and spatial evolution of thromboembolic stroke can be evaluated using an ADC map.

The rat model of cerebral infarction was established by Kudo and colleagues. This animal model of thromboembolism creates cerebral infarction predominantly in the territory of the middle cerebral artery, with a low mortality rate and excellent reproducibility. To establish a remote-controlled rat thromboembolic stroke from outside the magnet, we have modified this model and observed sequential changes in the hemispheric ischaemic lesion area caused by thromboembolism.

The objective of the present study was to determine the evolution of thromboembolic stroke in rats under general anaesthesia using ADC maps. We focused on the effect of the change in lesion volume at the early stage of stroke on total evolution of thromboembolic stroke, because recent therapy in acute stroke is based on reperfusion of the ischaemic legion leading to salvage of the ischaemic penumbra, smaller infarct size, and improved outcome.

Methods
This project was approved by, and followed the guidelines published by, the Animal Care and Use Committee of Tokyo Medical and Dental University.

Clot formation
Nine adult male Sprague–Dawley rats weighing between 330 and 400 g were used. Anaesthesia was induced with 4% isoflurane in a Plexiglas chamber. The concentration of isoflurane was then reduced to 1.5%, 1% lidocaine was locally injected for analgesia, and 0.25 ml of blood drawn from a left femoral vein and stored at room temperature for 48 h for clot formation. The clot was fragmented by extruding it twice through a 27-gauge needle. The clot and prepared for injection.

Animal preparation
The animals were again anaesthetized with 1.5% isoflurane, and 1% lidocaine was locally administered. Under an operating microscope, the bifurcation of the right common carotid artery was exposed through a midline neck incision. The external carotid artery was ligated just above its origin. A polyethylene catheter (PE-50) was then introduced into the right common carotid artery and advanced into the internal carotid artery. Two PE-50 catheters were introduced into a right femoral vein and a right femoral artery for drug infusion, arterial pressure monitoring, and arterial blood sampling, respectively. Physiologic saline solution at 4 ml kg$^{-1}$ h$^{-1}$ was administered i.v. throughout the study. After a tracheostomy, each rat was ventilated with 40% oxygen and the respiratory rate controlled to maintain $P_{a_{CO_2}}$ at approximately 40 mm Hg.

MRI
MRI was performed using a 4.7-T experimental imager/spectrometer system (Unity INOVA, Varian, Palo Alto, CA, USA) with a 330-mm horizontal bore magnet equipped with shielded gradients (maximal strength, 65 mT m$^{-1}$) and an 80-mm-ID quadrature detection coil. The slice plane was standardized by first obtaining preview spin-echo images. Spin-echo DWI (TR ms/TE ms=2000/64, 40-mm field of view, 128×128 matrix, 2-mm section thickness) consisted of nine coronal slices covering most of the rat brain. Diffusion weighting was applied along all three axes ($b = 0$ and 1100 s mm$^{-2}$) and a single DWI required an acquisition time of approximately 18 min. DWI with a $b$ value of 0 s mm$^{-2}$ corresponds to a T$_2$-weighted image (T2WI).

Pairs of DWIs with two $b$ values were used to generate maps of the ADC. ADC maps were calculated using the standard equation:

$$\text{ADC}=\ln\left(S_0/S_1\right)/(b_1 - b_0)$$

where $S_0$ and $S_1$ are the signals of the two DWI scans and $b_0$ and $b_1$ are 0 and 1100 s mm$^{-2}$.

Experimental protocol
After placing the animals supine in the probe, anaesthesia was maintained with 1.5% isoflurane in 40% oxygen. The rat’s body temperature was monitored with a rectal probe, and maintained at 37.5±0.5°C using a water-circulating heating pad during the MRI study. After animal positioning and shimming, a set of baseline DWIs was acquired. Homologous blood clots were introduced into the right internal carotid artery via the catheter. Sets of DWIs were acquired at 60-min intervals for 300 min beginning 40 min after clot injection ($T_{40}$, $T_{100}$, $T_{160}$, $T_{220}$, $T_{280}$, $T_{340}$). Arterial blood samples were collected at baseline, and at $T_{120}$ and $T_{240}$, and analysed on a laboratory blood gas analyser (STAT profile 5™ gas analyzer, NOVA Biomedical, USA). If mean arterial pressure (MAP) was reduced to less than 80 mm Hg, dopamine was administered i.v. to maintain the MAP over 80 mm Hg.

Definition of lesion volume
The ischaemic lesion was defined arbitrarily as the pixels in which the ADC decreased to less than 70% of the mean ADC of the ipsilateral hemisphere at baseline. Lesion area on an ADC map was calculated by multiplying the number of these pixels by the area of one pixel (approximately 0.1 mm$^2$). The lesion volume of ischaemic injury was determined by integration of the lesion areas for the nine slices. Two coronal slices which included the optic chiasm (slice A) and the area 4 mm caudal to it (slice B) were considered...
to represent the territory of the middle cerebral artery, and consequently the ischaemic lesion on slices A and B were divided by the hemispheric area to obtain the percentage lesion area, and results then compared with histopathology as described below. Sequential changes in the lesion volume on ADC maps were recorded.

Percentage change in the lesion volume (%ΔLV) was defined as the percentage difference between the lesion volumes at Tt and Tt+60 (t=40, 100, 160, 220, 280) to calculate the percentage increase or decrease during each MRI acquisition interval. The absolute values of %ΔLV were compared at different times to evaluate when the lesion volume changed (increased or decreased) markedly. Total percentage change in lesion volume (total %ΔLV) was defined to calculate percentage increase or decrease in lesion volume during the whole MRI study after thromboembolism. %ΔLV and total %ΔLV were defined and calculated as follows.

1. As DWIs were acquired at 60-min intervals, the %ΔLV between Tt and Tt+60 was defined as: %ΔLV (Tt+60/Tt) (%) = [(lesion volume (Tt+60) – lesion volume (Tt))/lesion volume (Tt)]×100.
2. Total %ΔLV was calculated as: Total %ΔLV (%) = [(lesion volume (T340) – lesion volume (T40))/lesion volume (T40)]×100.

Development of lesion area in specific regions
To analyse the development of an ischaemic lesion in some specific regions, cortex and caudate putamen on slice A and cortex and basal ganglia on slice B were selected. Then the lesion in these regions was subdivided into two parts according to their ADC values: severe lesion corresponds to ADC<400×10^{-6} mm² s⁻¹ and moderate lesion corresponds to the ischaemic lesion whose ADC value is equal to or more than 400×10^{-6} mm² s⁻¹ but less than 500×10^{-6} mm² s⁻¹. Sequential changes in lesion area were recorded and analysed.

Histopathology
After MRI studies, the animals were killed with an overdose of i.v. pentobarbital, and the brain was removed and sectioned coronally at 2-mm intervals. The sections most closely corresponding to slices A and B were selected and stained with a 2% solution of triphenyltetrazolium chloride (TTC) to identify infarcted tissue, and then photographed. Using a computer imaging system, the infarcted areas were divided by the ipsilateral hemispheric area to obtain the percentage hemispheric infarcted area.

Data analysis
All data are expressed as mean (SD). MAP, arterial blood gas data, plasma glucose concentration, rectal temperature, lesion volume (area) on ADC maps, and the absolute value of %ΔLV at different times were analysed using repeated measures analysis of variance followed by Tukey’s multiple comparison test. The relationship between %ΔLV during each MRI acquisition interval and the total %ΔLV was analysed using linear fitting. The relationship between lesion volume on the ADC map and the time after clot injection was analysed for each rat using linear fitting. The percentage lesion area based on the ADC map at T340 was compared with the TTC stained percentage hemispheric infarcted area using correlation analysis for slices A and B. Statistical significance was set at the P<0.05 level.

Results
No significant differences in MAP, heart rate, arterial blood gas, plasma glucose concentration, or rectal temperature at different time points throughout the study were observed (Table 1). Dopamine infusion was required in one rat to maintain MAP over 80 mm Hg.

ADC maps, DWIs, and T2WIs on slices A and B before and after thromboembolism in a representative rat are shown in Figures 1 and 2, respectively. DWI revealed a region of increased signal intensity in the territories of the right middle cerebral artery in all rats after clot injection. A clear reduction in the ADC value was evident in these regions after embolization. Lesion area was not evident on T2WI at T100 but apparent at T220 in both slices.

Mean lesion volumes did not differ significantly at the various time points. Lesion volume in individual rats showed some fluctuation during the MRI study (Figure 3). Lesion volume on the ADC map was larger at T40 than at T40 in eight of nine rats. There was a significant linear correlation between lesion volume on the ADC map and the time after clot injection.

Table 1 Haemodynamic indices, arterial blood gas, plasma glucose concentration, and rectal temperature at baseline, 100, 220, and 340 min after clot injection. Results are mean (sd). There were no significant differences in any index between any time points. MAP=mean arterial pressure; HR=heart rate

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>100 min</th>
<th>220 min</th>
<th>340 min</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>113 (8)</td>
<td>103 (18)</td>
<td>97 (20)</td>
<td>91 (18)</td>
</tr>
<tr>
<td>HR (beats min⁻¹)</td>
<td>329 (31)</td>
<td>363 (49)</td>
<td>393 (44)</td>
<td>361 (44)</td>
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<tr>
<td>PaO₂ (mm Hg)</td>
<td>198 (23)</td>
<td>191 (28)</td>
<td>188 (29)</td>
<td></td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>40.2 (5.8)</td>
<td>37.8 (3.9)</td>
<td>40.1 (4.6)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.39 (0.05)</td>
<td>7.38 (0.04)</td>
<td>7.36 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mg dl⁻¹)</td>
<td>207 (38)</td>
<td>161 (47)</td>
<td>176 (51)</td>
<td></td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>37.5 (0.3)</td>
<td>37.6 (0.1)</td>
<td>37.6 (0.3)</td>
<td>37.6 (0.2)</td>
</tr>
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relationship between lesion volume and the time after thromboembolism in five rats, and the mean (SD) slope and intercept were 0.43 (0.31) mm³ min⁻¹ and 243.9 (96.1) mm³, respectively.

%ΔLV during each MRI acquisition interval is shown in Figure 4. The absolute value of %ΔLV (T₄₀⁻₁₀₀) (22.7 (17.2)%) was two to three times larger than that at T₁₀₀⁻₁₆₀, T₁₆₀⁻₂₂₀, T₂₂₀⁻₂₈₀, and T₂₈₀⁻₃₄₀. It was significantly larger (P<0.05) than that at T₁₆₀⁻₂₂₀ (7.9 (7.2)%) and T₂₈₀⁻₃₄₀ (8.1 (8.5)%). Mean (SD) total %ΔLV was 41.6 (32.8)%. Correlation analysis revealed a significant linear correlation between %ΔLV (T₄₀⁻₁₀₀) and total %ΔLV (r=0.77, P<0.05) (Figure 5). No significant correlation was observed between %ΔLV at other time intervals and total %ΔLV.

Severe ischaemic lesions (ADC<400×10⁻⁶ mm² s⁻¹) significantly increased with time in the cortex on both slices. In the caudate putamen on slice A and basal ganglia on slice B, mean severe ischaemic lesions doubled during MRI

Fig 1 ADC maps, diffusion- and T₂-weighted images (DWI and T2WI) on slice A at baseline and 100, 220, and 340 min after thromboembolism.

Fig 2 ADC maps, diffusion- and T₂-weighted images (DWI and T2WI) on slice B at baseline and 100, 220, and 340 min after thromboembolism.
study, but there was no significant differences at the various time points (Figure 6). There was also no significant differences in moderate ischaemic lesions (400\(\times\)10\(^{-6}\) mm\(^2\) s\(^{-1}\)) at different time points in any selected region (Figure 7).

Mean (SD) percentage hemispheric infarcted area determined by TTC was 62.9 (17.8) and 56.8 (17.2)% on slices A and B, respectively. Postmortem TTC studies showed that all the animals had an infarction that correlated well to the hyperintense area on DWI and the area with a low ADC value on the ADC map. Significant correlation between the percentage hemispheric infarcted area and the hemispheric lesion area on the final ADC map for slices A and B was observed (Figure 8).

**Discussion**

We have assessed the evolution of thromboembolic stroke in rats using ADC maps. The lesion area was defined based on ADC maps for nine coronal slices and integrated to calculate lesion volume. Mean lesion volume tended to
increase with time although there was no significant difference at various time points and in individual rats there was some fluctuation. Lesion volume showed a maximal change at T_{40-100}, and a significant correlation was observed between the percentage change in lesion volume during that period (\%ΔLV (T_{40-100})) and the total change in lesion volume (total \%ΔLV).

Diffusion-weighted MR imaging has been well established as a reliable non-invasive method for early detection of cerebral ischaemic stroke.\(^{17,18}\) The principle of diffusion measurement is that the spatial location of each water molecule over time results in a signal loss or darkening of the images. In the present study, this tagging was achieved by a pair of very strong pulsed magnetic field gradients (diffusion gradients) which were added to a spin-echo sequence. The first of these diffusion gradients causes the spinning protons to fall out of phase with one another, and the second gradient rephases the protons completely with one another only if there has been no movement of the water molecules between the application of the two diffusion gradients. When there is movement of water, the protons are not brought back completely into phase by the second diffusion gradient and the signal attenuation will occur on DWIs. DWI can detect abnormalities within min after the onset of ischaemia in animal models of stroke.\(^{19-21}\) Before lesions are apparent on conventional images. The high signal intensity on the DWIs after clot injection may be explained as an ischaemic change as a result of thromboembolism. The infarct lesions revealed by TTC staining did not change\(^{32}\) on the first ADC map after thromboembolism but was detected later during the MRI study. Transient reduction in lesion volume (transient \%ΔLV) and percentage lesion area that was not visible on the ADC map on the early stage of the thromboembolism but was detected later during the MRI study may represent ‘penumbra’.\(^{30,31}\) Although cerebral perfusion was not evaluated in the present study, the most likely scenario is that cerebral blood flow in the ‘penumbra’ reduced slightly and the ADC decreased little or did not change\(^{32}\) on the first ADC map after thromboembolism. This hypoperfused tissue may be above the threshold for infarction, at least initially;\(^{33}\) however, it may be irreversibly injured on the time course of the MRI study.

Development of severe ischaemic lesions in the cortex on slices A and B may support our assumption that the enlargement of lesion volume may be most likely explained by an ischaemic penumbra. The lesion observed on the first ADC map after the thromboembolic stroke may be regarded as the central most severely ischaemic region (‘core’). The lesion area that was not visible on the ADC map on the early stage of the thromboembolism but was detected later during the MRI study may represent ‘penumbra’.\(^{30,31}\) Although cerebral perfusion was not evaluated in the present study, the most likely scenario is that cerebral blood flow in the ‘penumbra’ reduced slightly and the ADC decreased little or did not change\(^{32}\) on the first ADC map after thromboembolism. This hypoperfused tissue may be above the threshold for infarction, at least initially;\(^{33}\) however, it may be irreversibly injured on the time course of the MRI study.

Second, as shown in Figure 3, the lesion volume in individual rats showed some fluctuation during the MRI study. Transient reduction in lesion volume (transient recoveries of ADCs) in such animals may be, in part,
explained by recanalization and subsequent reperfusion (spontaneous reperfusion\(^8\), \(^9\)), a passage of emboli or a development of adequate collateral circulation. Disappearance of arterial cerebral occlusion is often seen clinically as a natural process of thrombus disintegration and endogenous thrombolysis. Although it is impossible to prove in the present study without measurement of cerebral perfusion, the proportional relationship between ADC and cerebral perfusion\(^2\) may indirectly support our assumption.

Third, pseudonormalization of the ADC may be another factor that affected the time course of lesion volume. In rodent stroke models, ADC begins to normalize at 24–48 h. This normalization does not imply tissue recovery but the beginning of tissue necrosis (pseudonormalization).\(^3\)\(^5\)\(^6\) As ADC is increased by vasogenic oedema,\(^2\) pseudonormalization is considered to be attributed to a balance of cytotoxic and vasogenic oedema. Lesion area was evident on T2WI at T\(_{220}\) in the present study, suggesting that vasogenic oedema occurred at that time. Lesion volume based on ADC maps may have been affected by pseudonormalization of ADC during the latter half of the time course of the MRI study; however, we assume the extent of underestimation of lesion volume because of pseudonormalization, if it occurred, may be small as the TTC stained infarcted area correlated well with lesion volume on the final ADC map.

The fact that the absolute value of %\(\Delta LV\) was largest at T\(_{40–100}\) may imply that lesion volume markedly increases or decreases at the early stage of thromboembolism. Irrespective of the mechanisms responsible for the sequential change observed in lesion volume, our data demonstrate that total %\(\Delta LV\) shows significant correlation to %\(\Delta LV\) at this period (T\(_{40–100}\)) but not thereafter. This suggests that enlargement or diminution of lesion volume during this period plays an important role in the total evolution of thromboembolic stroke in this model. Our results also suggest that only a small increase or even a decrease in lesion volume may be detected throughout the whole period of thromboembolism in rats in which the ischaemic lesion is partly salvaged at the early stage of thromboembolic stroke.

There is no absolute threshold of ADC decrease that predicts if a lesion is irreversible; maximal decreases in the ADC area are even reversible for short periods.\(^3\)\(^7\) This is why the threshold ADC values should be set arbitrarily. The mean (SD) ADC value on slices A and B at baseline were 718 (40)\(\times\)10\(^{-6}\) and 703 (44)\(\times\)10\(^{-6}\) mm\(^2\) s\(^{-1}\), respectively. We have set the threshold at 70% of the baseline, which would have been affects the study by Busch and colleagues.\(^3\)\(^8\) Morikawa and colleagues reported on the adverse effect of hyperglycaemia on transient focal brain ischaemia using the threshold ADC value of 500\(\times\)10\(^{-6}\) mm\(^2\) s\(^{-1}\).\(^3\)\(^9\) One may expect that −2 SD from the mean ADC at baseline would be better as the threshold ADC. The mean −2 SD ADC on slices A and B correspond to 583\(\times\)10\(^{-6}\) and 539\(\times\)10\(^{-6}\) mm\(^2\) s\(^{-1}\), respectively in the present study, which would have estimated the lesion volume (area) a little larger than our analysis.

In conclusion, the largest percentage change in lesion volume was found at the early stage of thromboembolism, and showed significant correlation with the total percentage change in lesion volume. Marked enlargement or diminution of the lesion volume may be observed at the early stage of thromboembolism. Thromboembolic stroke, which is partly salvaged at the early stage, may likely evolve to a lesser extent thereafter.

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