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IDENTIFICATION OF HYPERACUTE ISCHEMIC STROKE WITH A MORE HOMOGENOUS NATURE

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Abstract

Previous reports revealed that middle cerebral artery occlusion (MCAO) models in rats were very diverse in nature, and experimental stroke of a more homogenous nature had not been previously documented. This paper aims to present our novel observations of experimental stroke in rats with similar MRI characteristics after MCAO. Immediately after MCAO, 19 rats were placed into a 4.7 T MRI scanner, and diffusion weighted imaging (DWI) of axial and coronal planes was repeated every 10 minutes up to post-occlusion 115 minutes. Apparent diffusion coefficient (ADC) values of the ischemic lesions were calculated and compared to those of the unaffected contralateral hemispheres. Successful MCAO was defined when the whole left MCA territory showed ADC abnormality on DWI. Percentage of hemispheric lesion volume (% HLV), relative ADC value (rADC), and relative DWI signal intensity (rDWI) were serially evaluated for quantitative analysis of ADC-derived lesion characteristics. Successful MCA territorial infarction was induced in nine rats (9/19, 47.4%). In quantitative analysis of ADC-derived lesion characteristics, lesion volumes of seven rats (group 1) were very similar, but larger than those of the other two rats (group 2): % HLV of initial MRI = 45.4 ± 2.5 / 19.1 ± 6.6. rADCs and rDWIs of group 1 showed similar patterns of temporal change, which was different from those of group 2. Using prospective diffusion MRI after MCAO in rats, we identified territorial hyperacute ischemic lesions with similar MRI characteristics. This observation would contribute to the establishment of more homogenous rodent models for ischemic stroke translational research.

Keywords

• Acute stroke • Animal model • Diffusion weighted imaging • Focal cerebral ischemia • Magnetic resonance imaging • Rat

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Introduction

There have been numerous attempts to develop therapeutic pharmaceuticals for ischemic stroke but, despite initial success in animal models, all previous attempts have failed. Currently, intravenous administration of tissue plasminogen activator (IV-tPA) for early revascularization is the only effective clinical measure [1,2]. Methodological quality problems in preclinical research is one of many reasons for the discrepancies between experimental and clinical trials, as is the lack of cerebral blood flow (CBF) monitoring or validation of the ischemic infarction, which calls into question the reliability of the animal stroke model [1,3].

Several ischemic stroke models have been developed in order to investigate the pathophysiological mechanisms underlying brain injury after ischemic stroke and to develop effective therapeutic approaches to the disease. In general, experimental focal cerebral ischemia of middle cerebral artery occlusion (MCAO) models have been well-accepted for the similarities to ischemic stroke in patients. Currently, the intraluminal filament model of MCAO in rodents, first introduced by Koizumi *et al.* [4], is the most widely used animal model to study the pathophysiology of and therapeutic approaches to permanent and transient focal cerebral ischemia [5]. It is technically easy to induce MCAO and infarction and to control the duration of cerebral ischemia, but this model also has disadvantages in reproducibility and reliability. Although there have been efforts to increase the reproducibility of rat MCAO models [6-8], previous models presented very diverse results regarding cerebral infarction, i.e. location and volume of the ischemic lesions, temporal evolution of the stroke, and complication rate with the use of either sutures or macrospheres [9-14]. This diversity in rat MCAO models is caused by several variables, including shape and size of thread tips, mode of arterial obstruction, characteristics of the rats (such as strain, weight and vascular anatomy) [6-8,12,15].

Animal models with similar characteristics of cerebral ischemia/ infarction are essential for translational research to study focal cerebral ischemia [16,17]. Most often, successful MCAO in rats has been determined by behavioral assessment after the procedure [3,12]. It could be postulated that ischemic lesions with similar diffusion MRI characteristics are more homogenous in stroke nature; we tried to visualize the focal cerebral ischemia from the arterial occlusion using prospective, repeated diffusion weighted MRI (DWI) and observed the temporal evolution of acute ischemic lesions. This paper serves to report our novel observation of experimental stroke in rats with similar diffusion MRI characteristics after MCAO, and suggest the standard of selecting more homogenous rat MCAO models for translational research.

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Materials and methods

Experimental animals and MCAO

This study was approved by the institutional review board for the care and use of animals (KBSI-AEC 1203). Nineteen male Sprague-Dawley rats weighing 290-350 grams were subject to left MCAO using the intraluminal filament technique proposed by Koizumi *et al.* [4]. Anesthesia was induced by intramuscular injection of a mixture of zoletil and rompun (9:1, 200ul) for the procedure, and maintained with 1.5% to 2% isoflurane in a mixture of N_2O:O_2 (7:3) during MRI scanning. Respiratory rate was maintained at 20-80 / min. and the operation conducted on a heating pad.

The left common carotid artery (CCA) was exposed through an approximately 3cm midline incision in the supine position. The left CCA, external carotid artery, and pterygopalatine artery were ligated sequentially and the 4-0 silicon-coated nylon filament (the diameter of the distal 5mm tip was 0.15 - 0.25 mm) was introduced into the distal CCA and advanced until the tip had reached the proximal anterior cerebral artery and thus blocked blood flow to the MCA (Figure 1). If there was blood regurgitation from the puncture site of CCA, it was considered as incomplete arterial occlusion and another occluder with a larger distal tip was inserted instead. After the MRI, the animals were deeply anesthetized and euthanized.

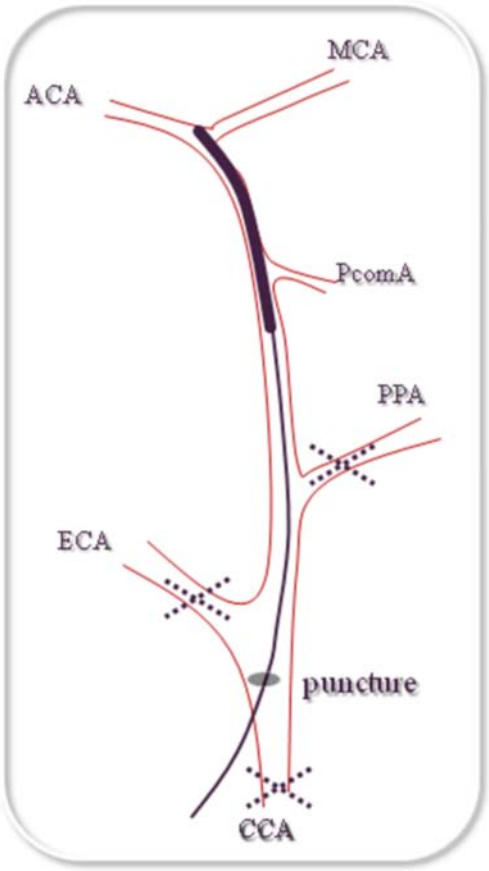


Figure 1. Diagram of intraluminal filament middle cerebral artery occlusion (MCAO) technique in rats. CCA, ECA, PPA were ligated and a silicon-coated nylon filament was inserted to occlude the MCA. ACA, anterior cerebral artery; CCA, common carotid artery; ECA, external carotid artery; PcomA, posterior communicating artery; PPA, pterygopalatine artery.

MRI data acquisition

Immediately after the arterial occlusion and still under general anesthesia, each animal was placed into a specially-designed harness. 4.7 T MRI scanner (BioSpec 47/40; Bruker, Ettlingen, Germany) and 72 mm RF volume coil was used for brain imaging. Diffusion weighted imaging (DWI) of axial and coronal planes was started in 15 minutes after stroke onset and repeated every 10 minutes up to post-occlusion 115 minutes (11 times) in the magnet. MRI parameters were as follows: b = 0 and 1000; FOV, 35 x 35 mm; matrix size, 128 x 128; TR/TE, 4000/60; NEX, 32; slice thickness, 1 mm. Immediately after each DWI, the apparent diffusion coefficient (ADC) values were determined in the ischemic lesions and compared to the unaffected contralateral hemisphere. ADC values were calculated by using the standard equation:

$$ADC = \ln(S0/S1)/(b1-b0)$$

(*S0* and *S1* are the 2 DWI signals at b = 0 and 1000, *b0* = 0, and *b1* = 1000)

Successful MCAO model

A successful MCAO was defined when the whole left MCA territory showed distinct ADC abnormality on three consecutive initial DWIs by visual inspection (Figure 2). Consensus was reached between three experienced, board-certified neuroradiologists. If an animal was classified as a case of unsuccessful MCAO, it was excluded from further study. Lesion volume and temporal changes of the lesional relative ADC (rADC) and rDWI were then analyzed to evaluate the MRI characteristics of ischemic damage.

Data analysis

Lesion volume measurement

Hemispheric lesion volumes (% HLV) were assessed by calculating and adding the area of abnormal ADC lesions in every sectional image with the equation of brain edema-corrected calculation proposed by Gerriets *et al.* [18]. Even though their measurement of %HLV was calculated from distortion-free T2WI and spin-echo diffusion-weighted image, echo-planar imaging (EPI) is widely used for DWI because of its low sensitivity to the motion-induced phase errors that occur during diffusion sensitization of the MRI signal [19]. To calculate the lesional

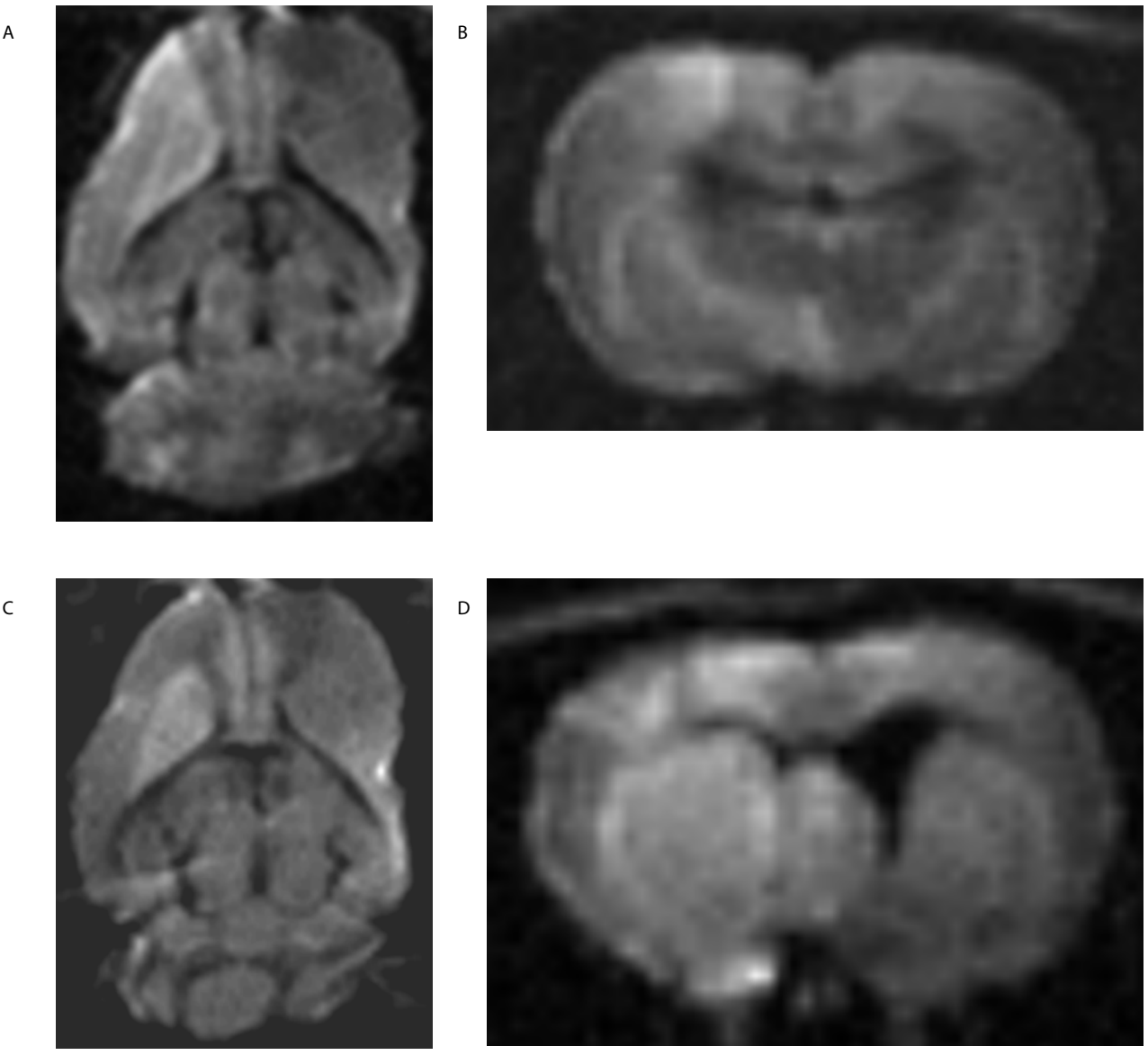


Figure 2. Successful MCAO with territorial infarction on DWI and a failed MCAO. (A, B) At post-occlusion 25 min, rat 7 shows diffusion abnormality in the whole left MCA territory in axial and coronal views. (C, D) On the contrary, rat 2 shows diffusion abnormality only in the left basal ganglia, classified as a failure.

area, image analysis software (ImageJ 1.46; a public domain Java image processing program, National Institutes of Health, Bethesda, MD, USA) was used after manually tracing the edges of the lesion and the ipsilateral left cerebral hemisphere (outline technique) on each diffusion-weighted image (Figure 3A).

$$\%\, HLVe = \frac{HVc - HVi + LVu}{HVc} \times 100$$

(% HLV_e, percentage of edema – corrected hemispheric lesion volume; HV_c and HV_i, contralateral and ipsilateral hemispheric volume; LV_u, uncorrected lesion volume)

Relative ADC value (rADC)

For the analysis of temporal changes of ADC values in the lesions, relative ADC values were calculated using Matlab (Mathworks, Natick, MA, USA) on the representative sectional images each time. Relative ADC value was the ratio between ADC values in two regions of interest (ROIs), one depicted by outlining the lesion and the other in the contralateral cerebral hemisphere comparably (Figure 3B).

Relative DWI signal intensity

To analyze temporal changes of DWI signal intensities, relative DWI signal intensities (rDWI) were calculated by a method similar to that used for calculation of rADC using ImageJ (Figure 3C).

Results

Successful territorial infarction was induced in nine rats (9/19). However, MCAOs in 10 rats induced small, non-territorial (n = 6) or non-ischemic lesions (n = 4) in the left MCA territory and were classified as cases of failure and exempted from the further imaging and analysis. There were no rats which had SAH or accompanying PCA territorial infarction in our cases. Occluders with a large distal tip (0.25 mm) were mainly used for successful MCAOs rather than smaller ones.

Lesion volume (% HLV)

Lesion volumes in % HLV changed very little in seven rats (group 1) from the initial MRI (post-occlusion 15 min) to the last and showed plateau with the time course, and those seven showed similar hemispheric lesion volumes (mean ± SD, 45.2 ± 2.3%) on the last MRI (post-occlusion 115 min). However the other two (rat 3 and rat 9, group 2) had smaller lesion volumes on initial MRIs (19.1 ± 6.6%) and were increased over time until the second MRI scan (post-occlusion 25 min). Eventually, they showed smaller lesion volumes (34.6 ± 0.7%) (Figure 4).

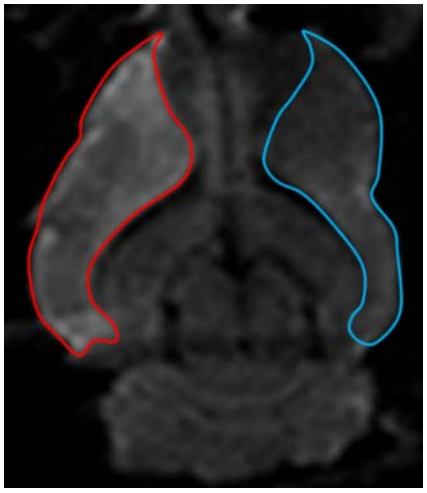
Relative ADC value (rADC)

In nine rats, ADC values of the ischemic lesions were significantly lower than those of contralateral hemispheres on initial MRI (rADC, 0.79 ± 0.08). Lesional ADC values in seven rats (group 1) showed a similar pattern of gradual decline in narrow ranges. The group 2 (rat 3 and rat 9), which had smaller lesion volumes, initially showed a different decline pattern until post-MCAO 35 min and 45 min, respectively (Figure 5).

Relative DWI signal intensity (rDWI)

The DWI signal intensities of the affected areas were higher than those of the matching areas in the contralateral hemispheres and increased gradually with the time course in all 9 rats. rDWIs of rat 3 and rat 9 were rather subtle (1.02 and 1.08, respectively) on the first MRI scan compared to those of the other 7 rats (1.4 ± 0.11 on initial MR) (Figure 6).

A



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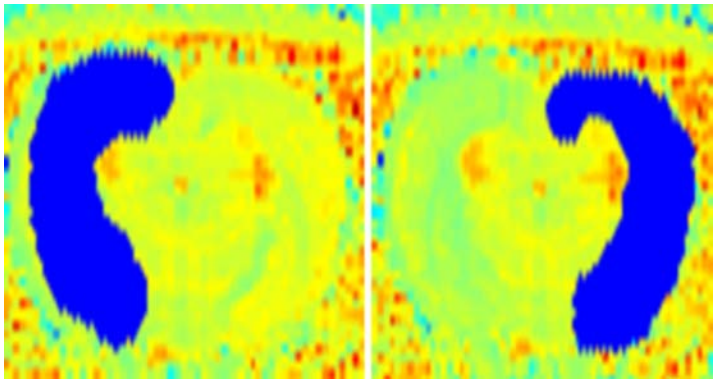

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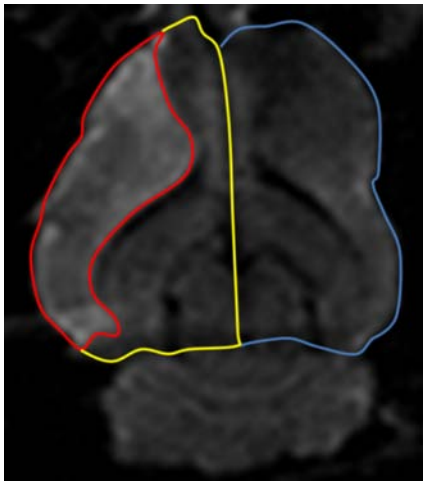

Figure 3. Methods of quantitative analysis. (A) Hemispheric lesion volumes were assessed by calculating and adding the area of abnormal ADC lesions in every sectional image, and were adjusted using brain edema-corrected calculation equation (Red ROI, *uncorrected lesion volume (LVu)*; yellow plus red ROI, *ipsilateral hemispheric volume (HVi);* blue ROI, *contralateral hemispheric volume (HVc)*). (B) Relative ADC value (rADC) was calculated by the ratio between two regions of interest (ROIs), depicted by outline technique in the lesion (left) and the other in the contralateral cerebral hemisphere (right) comparably. (C) Relative DWI signal intensity (rDWI) was calculated by a method similar to the one used for calculation of rADC (red, lesion ROI; blue, contralateral ROI).

Discussion

Among the nineteen rats, we were successful in inducing nine cases of focal cerebral ischemia in the left MCA territory. However, quantitative analysis of ischemic lesion volume, rADC and rDWI, showed that temporal changes of ADC-derived lesions of two rats in group 2 (rat 3 and 9) were different from those of the other seven in group 1. Thus, we were finally able to recognize seven out of 19 MCAO cases which had brain infarctions showing very similar MRI characteristics in lesion extent and temporal change of diffusion abnormality (Figure 7).

Until now, all the translational studies attempting to develop new therapeutic pharmaceutical treatment for ischemic stroke have failed. There have been several considerations and reflections regarding possible reasons for this "lack of translation" or "translational roadblock", that is, the physiological difference between rodents and humans, differences between experimental cerebral infarction and spontaneous brain infarction in patients, reasons attributable to the methodology of experimental studies and clinical trials, and so on [1,3,16,17]. Among these many reasons, the lack of a proper validation procedure of ischemic infarction leads to the fundamental matter of the reliability of the existing animal stroke models and this methodological quality problem caused futile attempts as in the clinical trial of NXY-059 [3]. Rat MCAO stroke models could be variable for diverse reasons including insufficient blocking of blood flow, body temperature loss, vasodilatation due to prolonged exposure to anesthesia, and so on [5,20], and this is the reason for which a proper validation procedure is needed. In our study, we did not use laser Doppler or perfusion MRI, but we hypothesized that repeated prospective DWI MRIs could validate successful MCA infarction. Therefore, we could exclude failed MCA territorial infarction owing to insufficient occlusion of the MCA. Though we could make homogenous MCAO models with a relatively low success rate, the model failure rate was 29% even in the expert group using MRI and MRA as a verifying method [12].

Among the nine successful MCAO models in rats, we observed two types showing different

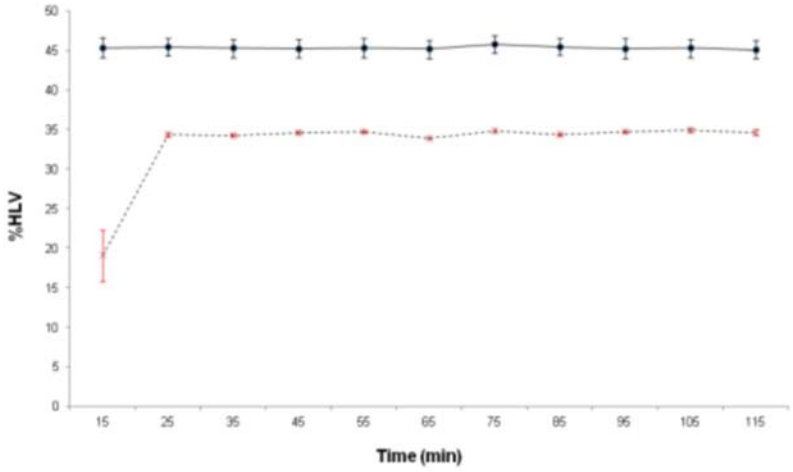

Figure 4. Temporal changes of percentage of hemispheric lesion volume (% HLV) in 9 rats. Seven rats in group 1 (solid line) show a different temporal pattern of lesion volumes compared to group 2 (Rat 3 and 9, dotted line). Infarction lesion volumes of group 1 are fixed from the initial MRI at post-occlusion 115 min and show plateau with the time course, but those of group 2 showed a rapid increase pattern until the second MRI scan (post-occlusion 25 min).

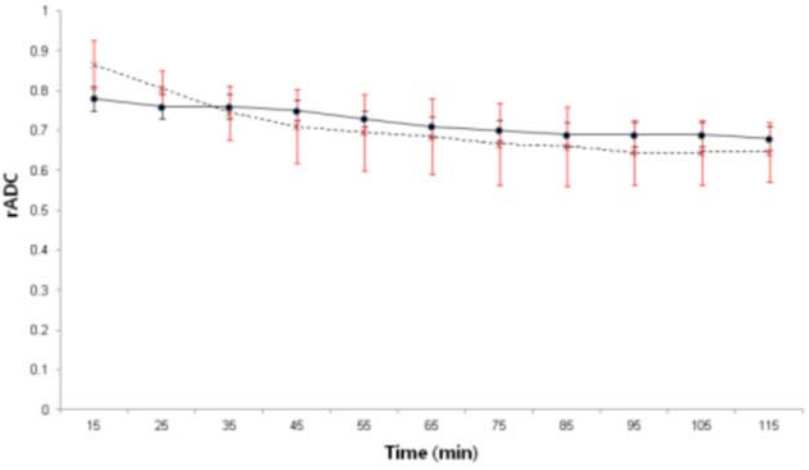

Figure 5. Temporal changes of relative ADC values (rADCs) in 9 rats. Seven rats in group 1 (solid line with black standard deviation bars) show a more stable pattern in narrow range from the initial MRI scan. Two rats in group 2 (Rat 3 & 9, dotted line with red standard deviation bars) shows a more rapid declining pattern for 45 min and remained stable thereafter.

spatiotemporal changes. While rats 3 and 9 (group 2) presented early rapid changes within half an hour of the characteristic in the ischemic lesions, lesion volumes and abnormal ADC values in seven rats (group 1) were stable in a narrow range over time from the first diffusion MRI. Though there have been several studies on murine focal ischemic stroke models using prospective serial MRI [10,11,13-15,21,22], those spatiotemporal changes observed in our seven rats (group 1) had not been reported to our knowledge. In previous studies, two different patterns of temporal changes of the ADC values after MCAO have been described: one is a gradual ADC decline pattern over time [14,15] and the other is acute ADC decline, which remained in a stable pattern thereafter [11,22]. In the former studies, the researchers analyzed temporal changes of ADC value up to 210 minutes using pixel-by-pixel analysis technique in the permanent intraluminal filament occlusion model group. The latter studies used ceramic macrospheres or intraluminal filament for permanent MCAO model and the ADC

values decreased rapidly for 45 minutes and remained stable thereafter [11,22]. In our study, group 2 (Rats 3 and 9) showed a similar pattern to the latter study, but the other seven rats in group 1 did not. We analyzed temporal changes of ADC value, setting the entire MCA territorial region as an ROI, but the other studies analyzed ADC changes setting small ROIs in the lesion, that might inadvertently mix and simplify the complex tissue characteristics and be an explanation accounting for the differences [23]. Nevertheless, two rats in group 2 (Rats 3 and 9) among 9 rats had whole MCA territorial infarcts and showed different spatiotemporal changes which were presumably related to a vascular anatomy with more collateral circulation to the affected hemispheres. If we were to evaluate a potential therapeutic agent for ischemic stroke using a permanent MCAO rodent model, experimental stroke of a more homogenous nature should be better recognized and selected for translational research.

DWI is generally considered the best non-invasive imaging method to detect acute cerebral ischemia and is well correlated with histological measurements of infarct size using either 2,3,5-triphenyltetrazolium chloride (TTC) or hematoxylin and eosin (H-E) staining [24-26], so we did not perform postmortem histological correlation to confirm the infarct size when DWI clearly showed the extent of the ischemic lesions. ADC changes in acute ischemic tissue are related with diffusion restriction of water molecules in the extracellular space caused by ion pump failure and a shift of extracellular water into intracellular components [27-31]. ADC value provided a valuable in vivo indicator of the risk of tissue injury in the case of permanent focal ischemia [32,33]. It is generally accepted that the intensity of the DWI signal (rDWI, 'brightness') relates to the proportion of damaged neurons [34]. Additionally, there have been studies comparing MRI characteristics to advanced histopathology other than TTC or H-E staining. Sequential ADC maps in conjunction with immunohistochemistry of heat shock protein 70 (HSP 70) after temporary focal ischemia in rats could distinguish infarct core from the ischemic [35]. Serial MRI perfusion with TdT-mediated dUTP-biotin nick-end labelling (TUNEL) assay in transient cat MCAO

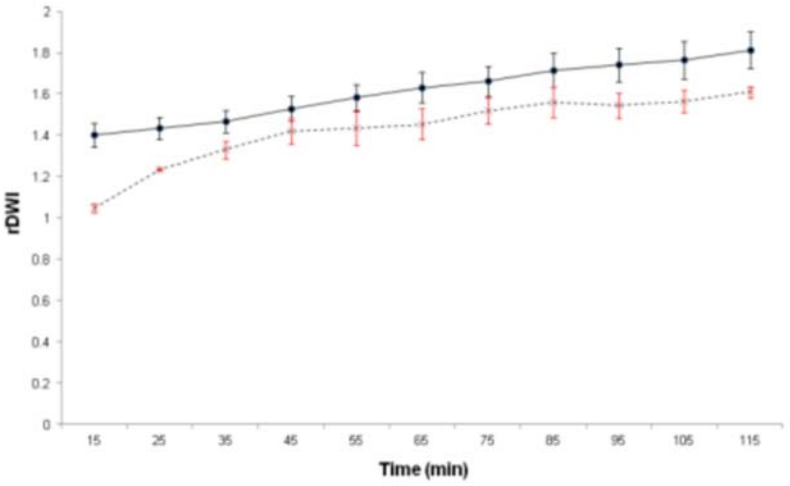

Figure 6. Temporal changes of relative DWI signal intensities (rDWIs) in 9 rats. Group 1 (solid line with black standard deviation bars) shows a gradual decline pattern from the initial MRI scan. Group 2 (Rat 3 and 9, dotted line with red standard deviation bars) shows a more rapid inclining pattern during 45 min and gradual incline pattern thereafter.

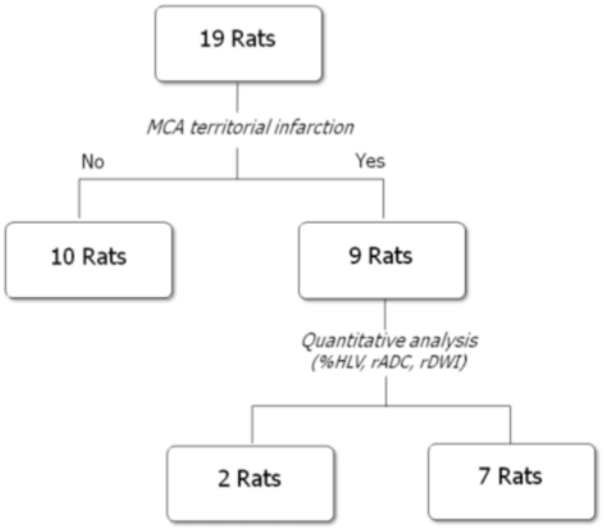

Figure 7. Flow chart identifying MCAO rat model of similar MR characteristics. Among nineteen rats, successful MCA territorial infarction was induced in nine rats. However, according to the quantitative analyses of lesion volume, rADC and rDWI, group 1 (seven rats) and group 2 (rat 3 and 9) showed different temporal changes of ADC-derived lesion from. Thus we were able to recognize seven of focal cerebral ischemia had brain infarctions showing similar lesion extent and temporal change of diffusion abnormality. Blood Flow Metab., 1998, 18, 367-375

demonstrated hyperperfusion induced cellular damage followed by reperfusion [36].

It may be assumed that if MCAO stroke models have more similar MRI characteristics, they are more homogenous. We focused on identifying territorial hyperacute ischemic lesions with similar MRI characteristics which may represent an acute stroke model of a more homogenous nature. To our knowledge there have been no previous studies with this concept of identifying a stroke model of a more homogeneous nature.

We used single parametric, echo-planar DWI MRI in evaluating the ischemic lesions.

While spin-echo DWI or multi-parametric MRI could be used for more accurate analysis of MRI characteristics, it is more time-consuming process. A rat brain is very small and it is thus difficult to define ischemic core and growing infarction respectively, so the whole ischemic area in an animal was outlined as a region of interest as in our study and the previous studies [10,37] and we then analyzed its MRI characteristics. However, temporal evolution of the infarction could also be evaluated by the voxel-by-voxel technique to measure different regional ADC values [15,23].

In conclusion, we could identify territorial hyperacute ischemic lesions with similar MRI characteristics and they may represent an experimental stroke of a more homogenous nature. Combining prospective repeated diffusion MRI with real-time analysis of diffusion characteristics would lead to a more homogenous model of hyperacute stroke, and we suggest that those rats should be used for preclinical translational stroke research.

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