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Time-dependent Changes of the Infarct Volume in a Rat Stroke Model: A Comparison of the Use of MRI and TTC-staining as Monitoring Tools

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ABSTRACT

Objectives : Serial changes of focal ischemic lesions as seen on magnetic resonance (MR) images and triphenyltetrazolium chloride (TTC)-stained samples of transient middle cerebral artery occlusion in a rat model were evaluated to investigate the natural course of the lesions and the feasibility of the use of each method as a monitoring tool. **Methods** : Transient middle cerebral artery occlusion (MCAO) was induced in fifteen adult female Sprague Dawley rats using the method of intraluminal vascular occlusion. Two hours after MCAO was induced, reperfusion was performed. Serial MR images were obtained and the volume of the brain infarct was estimated. For macroscopic and microscopic evaluation of the ischemic lesions, the ten animals were sacrificed at different times after MCAO. The rat brains were then removed and six coronal sections were made. Each section was incubated at 37 °C in 2% TTC solution for 15 minutes. **Results** : Post-ischemic injury evaluations that were made periodically for eight weeks revealed that the lesion volume as determined from T2 maps had reached a peak on the second day after ischemic injury and the volume decreased afterwards for one week; by the fourth week, the lesion volume again increased to stabilize initial lesion development. There were considerable discrepancies between the infarct area of the samples determined by TTC staining and the in vivo infarct area estimated from the MR images, especially for late stages. **Conclusion** : T2 map MR images, with a careful consideration of the natural course of infarction development, can provide an adequate and noninvasive means to evaluate the degree of ischemic injury under diverse experimental circumstances. **(Kor J Cerebrovascular Surgery 10(3):437-441, 2008)**

KEY WORDS : Rat stroke model · Infarct volume · MRI · TTC staining

Introduction

Animal models of stroke remain essential for understanding the mechanisms underlying brain tissue damage and the development of therapeutic strategies. Because of its noninvasive nature, its versatility, and its potential for imaging at relatively high spatial and temporal resolution, magnetic resonance (MR) imaging has evolved into a promising tool for biomedical experiments.[8] In adult rodents, MR images have been demonstrated as a useful method in the evaluation of acute ischemic studies as well as for the follow-up of stroke evolution.[10)12)18)] However, reported experiences of MR images for ischemic tissue injury were time-limited results, as only four week-long observation have been previously described.[10)11)13)24)] Recently, the need for suitable methods for the long-term evaluation of experimental ischemic changes in the rodent central nervous system has been raised because of the evolution of cell therapy strategy in which experiments need a more

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significant amount of time for evaluation. Although 2,3,5-triphenyltetrazolium chloride (TTC) staining is the most common method for differentiating viable tissue from infarcted tissue macroscopically, it is also questionable whether TTC staining can provide appropriate serial quantitative data of the long-term effects of ischemic tissue injury.[5] The objective of this paper is studying serial change of focal ischemic lesions in MR images and TTC-stained samples to investigate their natural course and feasibility as a monitoring tool for the purpose of more appropriate application of these methods to future experiments.

Materials and Methods

Materials

Fifteen adult female Sprague Dawley rats weighing 250-300 g were used. Animals were housed at a controlled temperature (21±1℃) with a 12 h light-dark cycle. Standard laboratory rat food pellets and tap water were supplied ad libitum.

Induction of stroke

Rats were anesthetized with an intraperitoneal injection of 1% ketamine (30mg/kg) and xylazine hydrochloride (4mg/kg). Their rectal temperature was controlled at 37℃ with heat blanket.

Transient middle cerebral artery occlusion (MCAO) was induced using a method of intraluminal vascular occlusion described elsewhere.[14] Briefly, a 2cm incision was made at the center of the neck, and the left common carotid artery, external carotid artery, and internal carotid artery (ICA) were exposed under an operating microscope. A length of 3/0 monofilament nylon suture with its tip rounded by heating near a flame was advanced into the lumen of the ICA until it blocked the origin of the middle cerebral artery. Two hours after MCAO, animals were reanesthetized, and reperfusion was performed by withdrawal of the suture until its tip reached the insertion point. Free access to food and water was allowed after recovery from anesthesia.

MR imaging

Serial MR images were taken daily from the day of MCAO to the fourth day after MCAO in one rat. Follow-up MR imaging was performed every week for eight weeks after MCAO in four rats. In ten rats, the MR imagings were taken weekly until they were killed. Rats were anesthetized with the intraperitoneal injection of 1% ketamine (30mg/kg) and xylazine hydrochloride (4mg/kg). A 1.5 T Advantage Horizon System (GE, Milwaukee, WI, USA) equipped with an actively shielded gradient coil, and wrist coil (GE), was used in this study. A solenoid-type volume coil 70mm in diameter for transmission and a surface coil 22mm in diameter for signal detection were combined perpendicularly. T2-weighted axial images were acquired with 3,500 ms repetition time (TR), 85 ms echo time (TE), 6 mm field of view (FOV), 256×160 resolution, and 2mm slice thickness at the center of the brain in the axial plane. Two-dimensional gradient-echo axial images were acquired with 300ms TR, 20ms TE, 20 degrees of flip angle, 60mm FOV, and 2 mm slice thickness at the center of the brain.

TTC staining

For the macroscopic and microscopic evaluation of ischemic lesions, the ten animals were killed two at a time in two days, and one, two, four, and eight weeks after MCAO. The rats were euthanized with an overdose of pentobarbital after MR imaging follow-up. Brains were then removed and six coronal sections (2mm thick slices from anterior 3.5mm to anterior 13.5mm) were made using a brain matrix (Agar Scientific, UK). Each section was incubated at 37℃ in 2% TTC solution for 15min. Then normal brain tissue stained brick red, while the area of infarct remained pale. The sections were then fixed with 10% formalin and processed with hematoxylin-eosin staining for microscopic examination.

Mesurements of the infarct volume

Morphometric measurements of the infarct volume of the rat brain were made using noncommercial software (Osiris, version 4.0). The volume of the brain infarct was estimated by measuring the volume of the high signal area ipsilateral to the MCAO in T2-weighted images and the pale area in TTC-stained brain sections.

Results

Infarct volume in MR images

Infarct volume changes in MR images showed same trends

with time in all rats. During the first week after MCA occlusion, the measured infarct volume in T2 maps emerged on the day of injury and reached a maximum in the second day(Fig. 1). From then on, infarct volume changes in T2 maps were expressed as a ratio of the volume measured weekly to the volume on the second day. Infarct volume continued to decrease after the second day of MCA occlusion until the first week and then gradually increased and reached the plateau after a month(Fig. 2).

Infarct volume in TTC stained samples

Measured infarct volumes in T2 maps were well correlated with the ischemic lesion seen by TTC staining during the first week. However, appropriate measurement of ischemic lesion volume could not be executed with samples obtained after one week, owing to the tissue loss at the infarct area(Fig. 3). Histological examination of lesion area in TTC staining confirmed the infarction.

Discussion

Within an ischemic lesion, MR parameters are time-dependent and spatially heterogeneous.[23] Many attempts have been tried with diverse sequences of MR images to monitor ischemic lesions in rodents for diagnostic assessments, tissue characterization, prognosis prediction, and estimation of therapeutic response.[2)4)7)11)18)19)23] T2 map MR images have long been considered as the gold standard measurement to identify ischemic lesions.[11] Although inaccuracy in the estimation of infarct volume is indicated during the early phase, T2 map MR images are known to provide the most consistent measurement corresponding with final lesion size.[10] It has been demonstrated that the size of the infarct does not increase further after 24 hours of ischemia, so the infarct volume on T2 maps, 24 hours after occlusion, can be considered as the final size.[21] Our result demonstrated that the lesion volume in T2 maps reached a peak on the second day after ischemic injury and decreased afterwards for up to one week after injury, and then increased again to stabilize by the fourth week after initial lesion development. The increase in T2 abnormality during the first two days may reflect an increase of brain edema.[10] Therefore, there is a possibility of overestimation of infarct volume in T2 maps during the early phase, even though the discrepancy may be insignificant when compared with that of TTC staining results shown in the present study.

Derugin et al.[7] suggested the usefulness of T2 and diffusion-weighted maps taken one week after temporary ischemic injury in neonatal rat brain as a surrogate measure of the long-term endpoint of stroke studies. Jiang et al.[11] showed significant correlations between MR image signatures at different time points and histopathological measurements of lesion area obtained at one week. We also observed a similar correspondence between T2 map lesions and TTC staining area one week after ischemic injury.

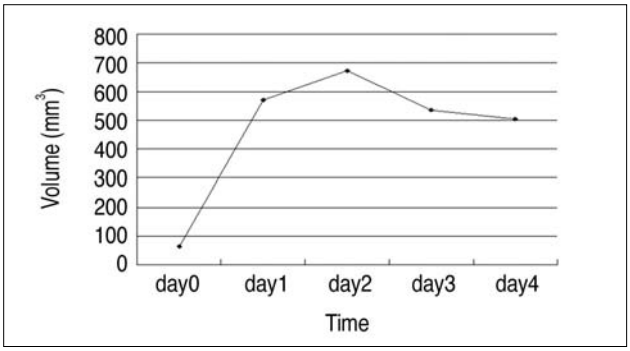

Fig. 1. Infarct volume changes in T2 map magnetic resonance images during the first week after MCA occlusion. The lesion started to develop on the day of injury (five hours after reperfusion), and reached maximum of its volume at the second day.

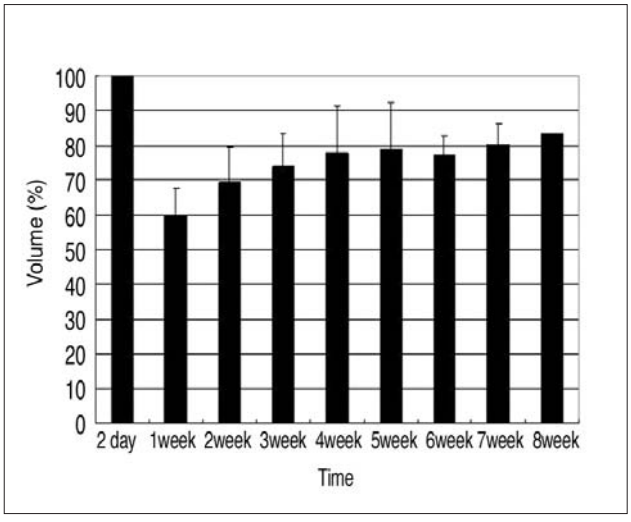

Fig. 2. Weekly changes of infarct volume in T2 map magnetic resonance images expressed as a ratio of the volume measured to the volume measured on the second day. Infarct volume continued to decrease after the second day of MCA occlusion until one week after the injury and then gradually increased to reach a plateau after four weeks.

However, infarct volume measured in T2 maps were then smaller than that of the final lesions, which could be attained at four weeks after injury. This result is inconsistent with previous rodent studies.[10)24)] Virley et al.[24)] showed in their MR images that infarct size in rats remained constant from seven to 28 days, by which time lesion development was considered to be complete. In a primate study, however, the phenomenon of late increase of infarct volume in MR images had been reported.[15)] Although the reason for these late changes is obscure, it can be partially explained by the "MRI fogging phenomenon" observed in human studies.[1)22)] During the early phase of stroke, magnetically active erythrocytes leaking out of pathologically altered blood vessels, the replacement of damaged tissue with scavenging, lipid-laden macrophages and a decrease in bulk water may lead to masking of the true extent of the lesion when examined using MRI in which lipid-filled macrophages would displace water, reducing the size of damage detected on the T2 map images.[15)22)] When the number of macrophages has returned to normal, the true extent of the lesion would again become apparent on T2W images.[15)] This "fogging effect" and the four-stage pathological process of neuronal death, inflammation, reorganization, and resolution that is thought to be complete by 30th days in rats can account for the course of infarct volume measured in T2 maps as shown in the present study.[6)]

Our result also demonstrated that there is a problem with the TTC staining method for volumetric analysis of infarct with time. There are considerable discrepancies between the infarct samples with TTC staining and in vivo infarct area estimated by MR images. Apart from the technical tissue loss problems in infarct area during the process, the measurement of the volume of ischemia by TTC staining disclosed many points of uncertainty.[3)] Many papers have indicated false positive or false negative detection of the infarction area by TTC staining owing to transient

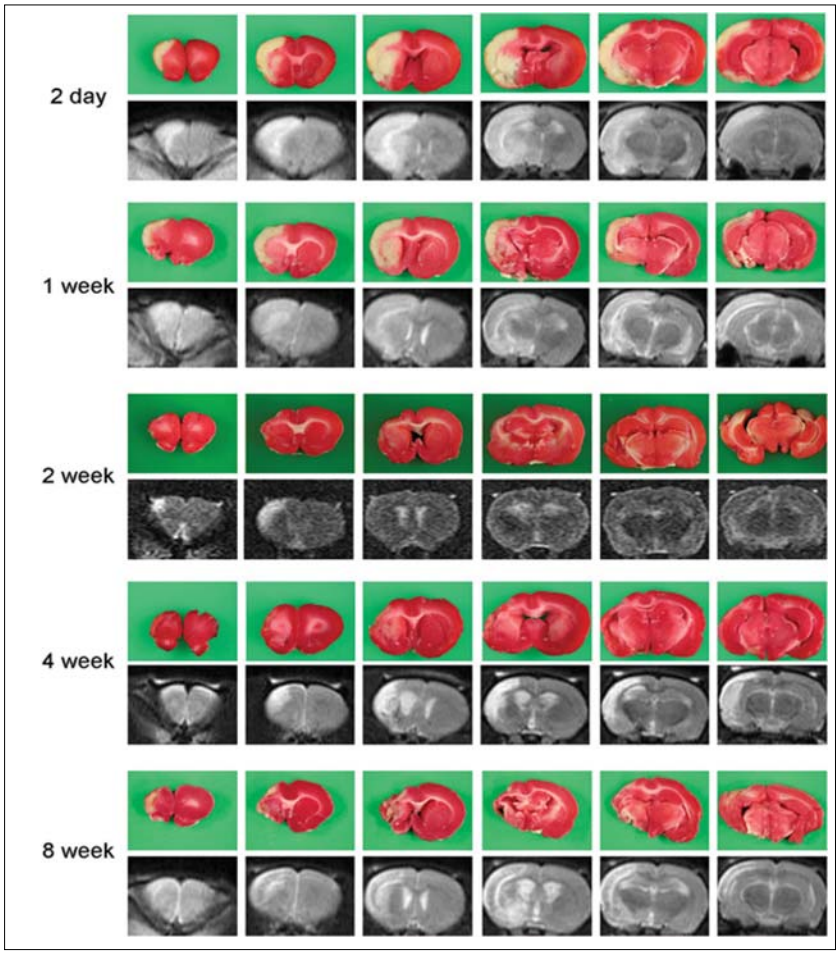

Fig. 3. Comparison of infarct area on T2 map magnetic resonance images (superior black-and-white pictures) and tissue samples stained using triphenyltetrazolium chloride (inferior color pictures). Although the lesion shows correspondence between two methods during the early phase, there are considerable discrepancies owing to the tissue loss at the infarct area in triphenyltetrazolium chloride stained samples.

inactivation of enzymatic activities or to different staining methods.[9)16)17)20)] These potential pitfalls cast some doubt on the validity of TTC staining as a reliable method for analysis of infarct volumes and imply that the results should be interpreted more cautiously.

Conclusion

Periodic evaluation for eight weeks after ischemic injury revealed that the lesion volume in T2 maps reached a peak on the second day after ischemic injury and decreased afterwards for one week, and then increased again to stabilize by the fourth week initial lesion development. There were considerable discrepancies between the infarct samples with TTC staining and in vivo infarct area estimated by MR images, especially in the late stages. Therefore, T2 map MR images, with careful consideration of the natural course of infarction development, can provide an adequate and noninvasive method to evaluate the degree of ischemic injury in diverse experimental circumstances.

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