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Therapeutic imaging window of cerebral infarction revealed by multisequence magnetic resonance imaging

An animal and clinical study☆

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Abstract

In this study, we established a Wistar rat model of right middle cerebral artery occlusion and observed pathological imaging changes (T2-weighted imaging [T_2WI], T_2FLAIR, and diffusion-weighted imaging [DWI]) following cerebral infarction. The pathological changes were divided into three phases: early cerebral infarction, middle cerebral infarction, and late cerebral infarction. In the early cerebral infarction phase (less than 2 hours post-infarction), there was evidence of intracellular edema, which improved after reperfusion. This improvement was defined as the ischemic penumbra. In this phase, a high DWI signal and a low apparent diffusion coefficient were observed in the right basal ganglia region. By contrast, there were no abnormal T_2WI and T_2FLAIR signals. For the middle cerebral infarction phase (2–4 hours post-infarction), a mixed edema was observed. After reperfusion, there was a mild improvement in cell edema, while the angioedema became more serious. A high DWI signal and a low apparent diffusion coefficient signal were observed, and some rats showed high T_2WI and T_2FLAIR signals. For the late cerebral infarction phase (4–6 hours post-infarction), significant angioedema was visible in the infarction site. After reperfusion, there was a significant increase in angioedema, while there was evidence of hemorrhage and necrosis. A mixed signal was observed on DWI, while a high apparent diffusion coefficient signal, a high T_2WI signal, and a high T_2FLAIR signal were also observed. All 86 cerebral infarction patients were subjected to T_2WI, T_2FLAIR, and DWI. MRI results of clinic data similar to the early infarction phase of animal experiments were found in 51 patients, for which 10 patients (10/51) had an onset time greater than 6 hours. A total of 35 patients had MRI results similar to the middle and late infarction phase of animal experiments, of which eight patients (8/35) had an onset time less than 6 hours. These data suggest that defining the "therapeutic time window" as the time 6 hours after infarction may not be suitable for all patients. Integrated application of MRI sequences including T_2WI, T_2FLAIR, DW-MRI, and apparent diffusion coefficient mapping should be used to examine the ischemic penumbra, which may provide valuable information for identifying the "therapeutic time window".

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Key Words

ischemic penumbra; therapeutic time window; diffusion-weighted MRI; apparent diffusion coefficient; intracellular edema; cerebral infarction; MRI; therapeutic imaging window; neural regeneration; neuroimaging; middle cerebral artery occlusion

Research Highlights

(1) The main pathological change of the ischemic penumbra was intracellular edema in a rat model

of middle cerebral artery occlusion.
(2) Imaging manifestations of clinical cases were classified according to imaging characteristics of middle cerebral artery occlusion animals at different phases, and showed that imaging manifestations are not consistent with infarction time. Thus, the use of 6 hours after infarction as the "therapeutic time window" remains controversial.
(3) Integrated application of T_2WI, T_2FLAIR, diffusion-weighted MRI, and apparent diffusion coefficient mapping should be used to reveal the ischemic penumbra, which can provide valuable information for identifying the "therapeutic time window".

Abbreviations

ADC, apparent diffusion coefficient; DWI, diffusion-weighted imaging; DW-MRI, diffusion-weighted MRI

INTRODUCTION

The incidence of cerebral infarction is rising with the increasing age of the world's population. Cerebral infarction can often lead to high levels of mortality, particularly when not treated effectively, and has a serious effect on human health[1-2]. Infarction can also lead to irreversible brain damage. Thus, it is critical to diagnose and effectively treat cerebral infarction. The pathological changes that result from cerebral infarction are a gradually developing process. The most effective treatment involves saving the ischemic penumbra within a specified amount of time after infarction[3]. To effectively prevent ischemic tissues from necrosis, the ischemic penumbra must be effectively converted into normal brain tissues in a timely manner. The most effective method for reversing the impact on ischemic penumbra is the application of thrombolytic therapy within the "therapeutic time window"[4]. The therapeutic time window refers to a time period after infarction during which the ischemic penumbra can be recovered. The therapeutic time window exhibits obvious dynamic changes and differing characteristics for each individual case[4-5]. The ischemic penumbra is typically defined as the mismatch area between perfusion-weighted imaging and diffusion-weighted imaging (DWI), and a high DWI signal represents a zone of irreversible brain damage[4]. The "therapeutic time window" for cerebral infarction is generally considered the time period less than 6 hours after infarction[4-5]. However, these standards have been questioned[6]. A previous study showed that there are individual differences in cerebral perfusion, and there is a "therapeutic physical window" in each individual[7].

However, there are no effective and objective imaging indicators for determining this period for effective clinical treatment. Animal experiments have also demonstrated that pathological changes in early cerebral infarction tissues involve intracellular edema and high signal on diffusion-weighted imaging[8-10]. However, the MRI characteristics of the penumbra remain unclear.
In the present study, we established a rat model of middle cerebral artery embolism reperfusion to simulate the penumbra, and examined the penumbra–"therapeutic image window" by MRI to provide a visual imaging method for early treatment and effective evaluation of cerebral infarction.

RESULTS

Animal experiments

Quantitative analysis of experimental animals

A total of 78 healthy Wistar rats were randomly divided into three groups: control group (n = 6), occlusion group (n = 36) and reperfusion group (n = 36). Occlusion group animals received occlusion of the right cerebral middle artery. In the reperfusion groups, an external light nylon fishing line in the right carotid artery of embolized rats was pulled until a slight resistance was felt, and reperfusion was performed for 1 hour. Control group animals did not receive carotid artery thrombosis, while all other procedures were the same as those in the occlusion groups. Both occlusion and reperfusion groups were divided into six subgroups, with 0.25, 0.5, 1, 2, 4 and 6 hours of middle cerebral artery occlusion (n = 6 per subgroup). Rats lost due to failed cerebral ischemia induction were supplemented with new animals, and a total of 78 rats were included in the final analysis.

MRI imaging of rat infarcted tissues at various infarction and reperfusion time points

At each time point, the control group signals for the T2-weighted imaging (T_2WI), T_2FLAIR, and DWI images showed no abnormalities. In the occlusion group, five rats (83.3%) showed a high signal in the right basal ganglia region at 0.25 hours, while all rats (100%)

demonstrated a high signal in the same site after 0.5 hours; the ΔDWI increased gradually with time, with a rapid increase from 0.25–2 hours, and then a slow increase from 4–6 hours. The Δapparent diffusion coefficient (ΔADC) demonstrated a rapid linear decline from 0.25–2 hours, and there was a slow recovery from 4–6 hours. Two rats in the reperfusion group (33.3%) showed high signal in the right basal ganglia region at 0.25 hours, and ΔDWI within 2 hours was significantly lower than that of the occlusion groups for the corresponding period (t = 22.69; P < 0.01). However, at 4–6 hours, there was no difference in ΔDWI of the reperfusion groups compared with the occlusion groups (t = 3.32; P > 0.05). ΔADC in the reperfusion groups were significantly higher than the occlusion groups at 0.25–2 hours (t = 18.36; P < 0.05), while there were no differences at 4–6 hours (t = 4.28; P > 0.05).

On T_2WI and T_2FLAIR images, approximately 50% of animals demonstrated a high signal at 2 hours after occlusion, while at 4 hours, 100% of animals exhibited a high signal. There were no differences in the positive rates of infarction at 6 hours between the three imaging methods (T_2WI, T_2FLAIR, and DWI) (X^2 = 3.91; P > 0.05; Table 1, Figures 1–3). Figures 1–3 show the MRI features of early cerebral infarction, with a high signal on DWI, a low ADC signal, and no abnormalities on T_2WI and T_2FLAIR. The MRI features of middle and late cerebral infarction included isointense or high (mixed) signal on DWI, low or high signal on ADC, and high signal intensity on T_2WI and T_2FLAIR.

Table 1 High MRI signal in the right basal ganglia region of occlusion and reperfusion groups (n)

Group	MRI	Time of occlusion (hour)					
		0.25	0.5	1	2	4	6
Occlusion	DWI	5	6	6	6	6	6
	T_2FLAIR	0	0	0	3	5	6
	T_2WI	0	0	0	3	5	6
Reperfusion	DWI	2	3	3	3	4	6
	T_2FLAIR	0	0	0	2	4	6
	T_2WI	0	0	0	2	4	6

n = 6 per group per time point. DWI: Diffusion-weighted imaging.

Pathological changes in rat infarcted tissues at different cerebral infarction and reperfusion time points

Control group animals showed normal brain integrity and astrocyte morphology. By contrast, at 0.25 hours after middle cerebral artery occlusion, a small number of swollen glial cells with a round appearance, enlarged soma, and a light eosinophilic cytoplasm were observed in the right basal ganglia and the adjacent frontal cortex by light microscopy (Figure 4A).

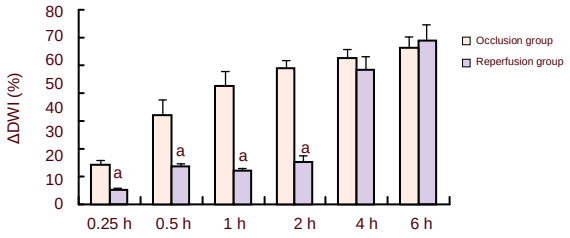

Figure 1 Comparison of Δdiffusion-weighted imaging (ΔDWI) between occlusion and reperfusion groups.

Data are expressed as mean ± SD (n = 6 per group per time point). [a]P < 0.01, *vs*. occlusion group (paired t-test). ΔDWI = (infarct area/total area of ipsilateral hemisphere) × 100%. h: Hours.

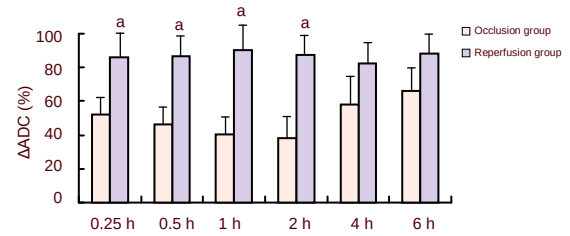

Figure 2 Comparison of Δapparent diffusion coefficient (ΔADC) between occlusion and reperfusion groups.

Data are expressed as mean ± SD (n = 6 per group per time point). [a]P < 0.05, *vs*. occlusion group (paired t-test). ΔADC = (infarct ADC/contralateral mirror area ADC) × 100%. h: Hours.

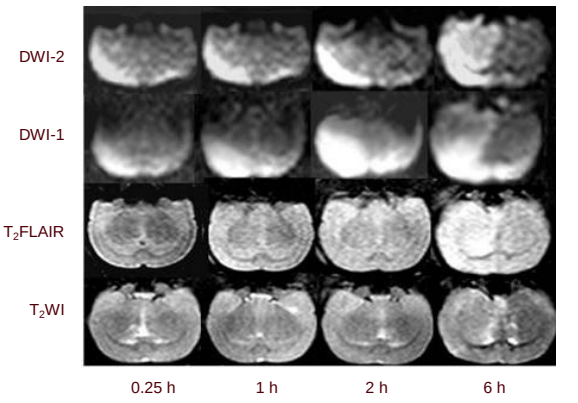

Figure 3 T_2WI, T_2FLAIR, and diffusion-weighted imaging (DWI) images of occlusion and reperfusion groups at different time points.

DWI-1 reflects the occlusion groups, and DWI-2 reflects the reperfusion groups. Occlusion groups had a high DWI signal from 0.25 hours, ΔDWI increased with time, and rapidly increased from 0.25–2 hours. The ΔDWI in the reperfusion groups at 2 hours was less than that in the corresponding occlusion groups. However, there were no differences at 6 hours; T_2FLAIR had a high signal at 2 hours, T_2WI had a high signal at 6 hours, and there were no differences in positive rates at 6 hours between the three methods (T_2WI, T_2FLAIR, and DWI). h: Hours.

At the same time, mitochondrial swelling, vacuole-like changes, expanded endoplasmic reticulum, nuclear swelling, and chromatin margination were observed by electron microscopy in the same tissues (Figure 4B).

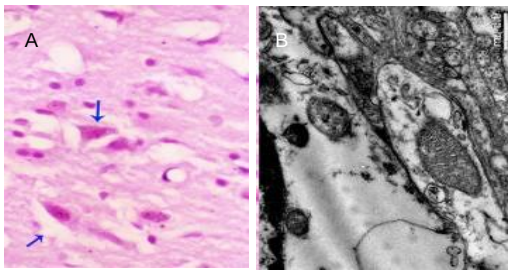

Figure 4 The pathological changes of the right basal ganglia in rats at 0.25 hours of middle cerebral artery occlusion. Arrows: Intracellular edema.

(A) By light microscopy, the glial cells in the ischemic area were swollen, and there was slightly stained eosinophilic cytoplasm, with a rounded shape, and an increase in volume (hematoxylin-eosin staining, × 1 000).

(B) By electron microscopy, there was evidence of mitochondrial swelling, crest deformation, and endoplasmic reticulum vacuolization (transmission electron microscopy, × 6 000).

At 1 hour, dark stained nuclei and eosinophilic changes in parts of the cytoplasm (red neurons) were observed by light microscopy, and organelle swelling of the entire membranes was observed by electron microscopy. At 2 hours, nuclear condensation and glial cell swelling became more evident, with light transmission appearing around the cells and a decrease in intercellular space; however, the cell membrane remained intact. At 4 hours, endothelial cells appeared swollen, the space between blood vessels and cells expanded, glial cell membranes became swollen and thick, vessels were compressed and deformed, and a light red mesh-like structure emerged in the tissue space, indicative of angioedema. There was also evidence of partly ruptured cell membranes, karyolysis, chromatin margination, appearance of medullary structures within the cytoplasm, and damage to the blood-brain barrier (Figure 5).

At 6 hours, these changes (angioedema) continued to progress, with appearance of tissue and glial cell necrosis. The reperfusion group exhibited significantly reduced cellular edema compared with the occlusion group at each time point, particularly within 2 hours (Figure 6), although the decrease of angioedema was increased.

Clinical results

Quantitative analysis of subjects

A total of 86 patients with cerebral infarction were included in the final analysis.

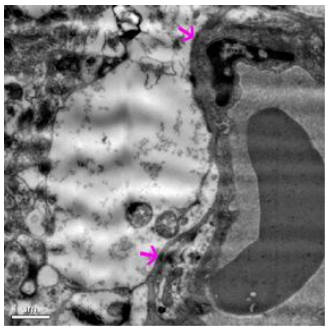

Figure 5 Pathological changes of the right basal ganglia in rats after 4 hours of middle cerebral artery occlusion (transmission electron microscopy, × 6 000).

There was evidence of rupture of part of the cell membrane, nuclear fusion, and blood-brain barrier (arrows) damage, with signs of angioedema.

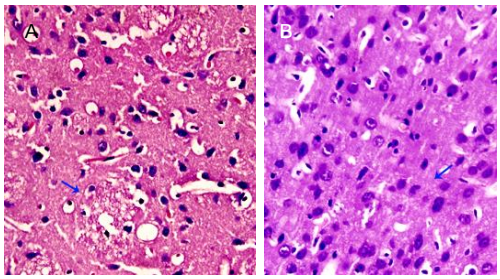

Figure 6 Pathological changes of occlusion and reperfusion groups after 2 hours of middle cerebral artery occlusion (hematoxylin-eosin staining, × 400).

(A) In the occlusion group, there was evidence of significant intracellular edema (arrow) and positively stained cell nuclei.

(B) The intracellular edema (arrow) of reperfusion group was significantly reduced.

Relationship between image manifestation and infarction time

A total of 86 of the clinical cases exhibited similar MRI signs of cerebral infarction as those observed in animal experiments. There were 51 cases with the same MRI signs (including a high DWI signal and a low ADC signal, with normal T_2WI and T_2FLAIR signals). This group of 51 clinical cases included 41 cases for which the onset times were less than 6 hours (80.4%), while the remaining 10 cases had an onset time greater than 6 hours. Some clinical cases also demonstrated MRI characteristics that were similar to those of middle and late cerebral infarction characteristics, including a high or mixed DWI signal and a low or high ADC signal. T_2WI and T_2FLAIR results showed a high signal in 35 cases, which included 27 cases with an onset time greater than 6 hours (77.1%), and eight cases with an onset time less than 6 hours (Table 2, Figures 7–9).

Table 2 Performance and onset time of clinical cases by MRI (*n*)

Onset time	Early cerebral infarction			
	T_2WI (normal)	T_2FLAIR (normal)	DWI (high)	ADC (low)
Less than 6 hours	41			
Greater than 6 hours	10			
Sum	51			
Onset time	**Middle, late cerebral infarction**			
	T_2WI (high)	T_2FLAR (high)	DWI (mix)	ADC (mix)
Less than 6 hours	8			
Greater than 6 hours	27			
Sum	35			

ADC: Apparent diffusion coefficient; DWI: diffusion-weighted imaging.

DISCUSSION

Pathological basis of early cerebral infarction and penumbra

The pathology of cerebral infarction develops gradually according to a very specific process. Experimental studies have shown that timely reperfusion in the early stage of cerebral infarction can effectively reduce the final infarction size, and the earlier the treatment onset, the smaller the final infarction size. Therefore, timely diagnosis of early cerebral infarction can greatly improve the overall outcome for patients who suffer from cerebral infarction. A study provided the evidence of an ischemic penumbra in the early recovery period from cerebral infarction[11], which has been found to represent a region of reversible loss of brain cells function, but with maintenance of cellular structure[12]. Thus, the key to effective clinical treatment is the preservation of the penumbra and its transformation into normal tissue. However, there is a limited time in which the penumbra may be effectively saved, and characteristics of penumbra development and organization differ in individual subjects. Furthermore, despite the various theories of optimal penumbra treatment[13-15], the systematic pathological description of the penumbra remains unclear.

In the present study, we demonstrated that the main change of intracellular edema could be observed within 2 hours after cerebral infarction in rats; intracellular edema and angioedema were the predominant changes observed between 2–4 hours after cerebral infarction induced by middle cerebral artery occlusion in rats, while by 4–6 hours, the main changes involved angioedema and cell necrosis. Based on these results, we artificially divided the pathological development of cerebral infarction into the following time segments and corresponding pathological changes: early cerebral infarction (less than 2 hours, intracellular edema); medium cerebral infarction (2–4 hours, mixed edema); and late cerebral infarction (4–6 hours, angioedema).

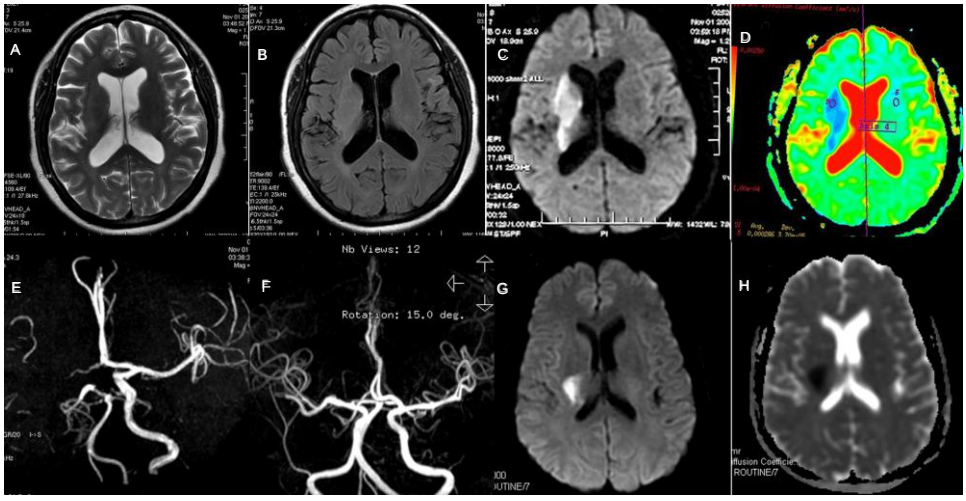

Figure 7 A female patient, aged 50 years, with cerebral infarction onset time greater than 6 hours.

(A–E) T_2WI, T_2FLAIR, diffusion-weighted imaging (DWI), apparent diffusion coefficient (ADC) maps, and magnetic resonance angiography (MRA) at the onset time. (F–H) ce-MRA, DWI, and ADC maps after treatment. (A, B) Not exceptional. (C) High signal at the right side of the basal ganglia. (D) Low signal at the right side of the basal ganglia. (E) Occlusion at the right middle cerebral artery, suggesting early cerebral infarction with a penumbra (an indication of the clinical “therapeutic time window”). After 3 days of thrombolytic therapy, the right middle cerebral artery was recanalized upon reexamination (F), and the DWI high signal area was significantly reduced (G), while ADC was recovered (H).

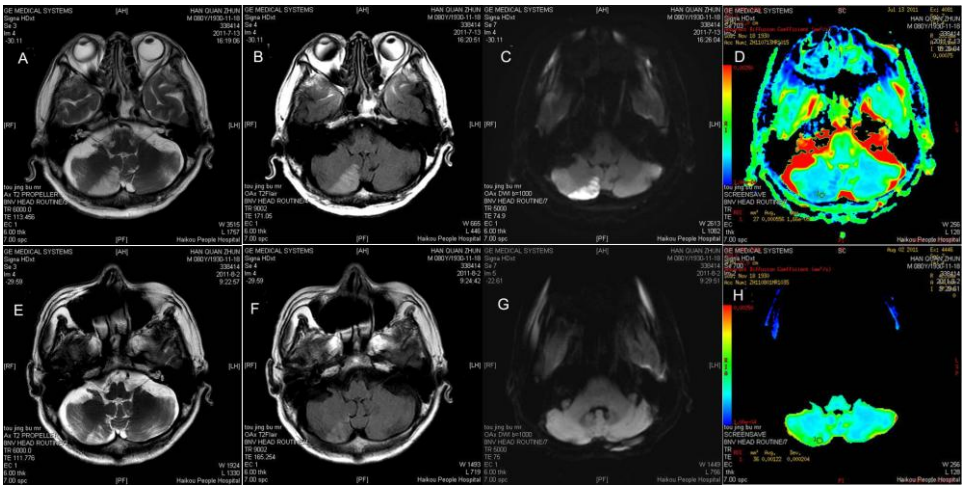

Figure 8 A male patient, aged 80 years, with cerebral infarction onset time less than 6 hours.

(A–D) T_2WI, T_2FLAIR, diffusion-weighted imaging (DWI), and apparent diffusion coefficient (ADC) maps at the onset time. (E–H) T_2WI, T_2FLAIR, DWI, and ADC maps after treatment. (A–C) High signal in the right cerebellar hemisphere. (D) ADC values of the lesion area were decreased, suggesting a middle cerebral infarction, with part of the penumbra remaining. After 7 days of thrombolytic therapy, high signal of the right cerebral hemisphere was reduced upon reexamination (E–G), and ADC had recovered (H).

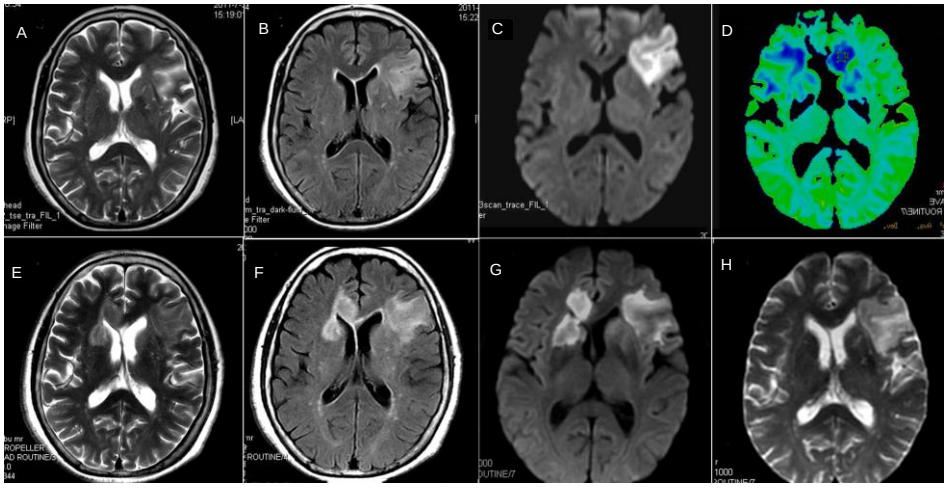

Figure 9 A female patient, aged 66 years, with cerebral infarction onset time less than 6 hours.

(A–D) T_2WI, T_2FLAIR, diffusion-weighted imaging (DWI), and apparent diffusion coefficient (ADC) maps at onset time. (E–H) T_2WI, T_2FLAIR, DWI, and ADC maps after treatment. (A–C) High signal in the left frontal lobe. (D) Low signal of the left frontal lobe, suggesting middle cerebral infarction, with part of the penumbra. After 10 days of thrombolytic therapy, the high signal area of left frontal was increased, and there was evidence of a high signal at the nearside of the right ventricle anterior horn (E–G), while ADC increased (H).

To confirm the existence of the penumbra, we also performed reperfusion experiments, and found that in the early infarction period (0.25–2 hours), there was a significant reduction in cell edema, while in the middle infarction period (2–4 hours), cell edema had improved slightly, but angioedema had worsened. Finally, in the late infarction period, angioedema significantly increased, and there was evidence of cell necrosis and hemorrhage.

These data suggest that the penumbra exists only in the early and middle stages of cerebral infarction. Furthermore, intracellular edema was the major pathological change observed during this time. Thus, this period likely represents the “therapeutic time window” for effective clinical treatment. In the middle stage of cerebral infarction, part of the penumbra was still present in some cases. However, there was severe angioedema at this time after reperfusion, with evidence of

reperfusion injury. In the late stage of cerebral infarction, there was no penumbra present and worsening of angioedema after reperfusion, suggesting a high risk for reperfusion injury in the late infarction period. This provides further confirmation and refinement of our previous report[8].

Recognition of diagnostic values of (diffusion-weighted MRI) DW-MRI for the early cerebral infarction

At present, DW-MRI is the most effective *in vivo* imaging technique to detect water diffusion, and ADC values can accurately reflect the degree of cellular edema[9, 14]. Thus, DW-MRI is accepted as the best method for early diagnosis of cerebral infarction[16-19]. However, the underlying causes of a high DWI signal in pathological tissues remain controversial, and have been suggested to represent both the irreversibly damaged area of brain tissue and the penumbra. Furthermore, the penumbra areas have been suggested to represent the mismatch areas of perfusion-weighted imaging and DWI[19]. In the present study, we observed a linear increase in the high signal area between 0.25–2 hours (early infarction period), and a linear decrease in ADC values. However, during this time period in the reperfusion groups, the high DWI signal in this area was significantly reduced, and the ADC values returned to normal levels. Simultaneously, there were no abnormalities on T_2WI and T_2FLAIR signals. At 4–6 hours (late infarction periods), the high signal area of the occlusion groups increased slowly, and the ADC values rebounded. However, in the reperfusion groups, the high DWI signal area at this stage rapidly increased, and the ADC values continued to rise. Furthermore, there were high T_2WI and T_2FLAIR signals at this stage.

From these data, we classified the “early” cerebral infarction as the “therapeutic time window”, as the high DWI signal, low ADC signal, and normal T_2WI and T_2FLAIR signals were all indicative of the penumbra. For classification of the middle cerebral infarction, in which there may be part of a penumbra, and which includes the final limit of the “therapeutic time window”, we observed a high DWI signal, low ADC signal, and T_2WI and T_2FLAIR exhibiting a high signal. For the late cerebral infarction, when there was no penumbra, and in which there was a strictly controlled “therapeutic time window” in clinical studies, we observed a mixed DWI signal, high ADC signal, and high T_2WI and T_2FLAIR signals.

The main pathological changes during the early stage of cerebral infarction involved intracellular edema (penumbra), in which only swollen and enlarged glial cells were observed, but no change in the total water content of brain tissue, reflecting the high DWI signal, reduced ADC, and no signal abnormality on T_2WI and T_2FLAIR. However, in the middle and late cerebral infarction periods, there was evidence of angioedema and an increase in the total water content of brain tissue, particularly in the late period. Furthermore, a large number of cells had ruptured and were necrotic, and tissue T_2 and ADC values increased. During this time, T_2WI and T_2FLAIR results showed a high signal, while the high DWI signal at this time was mostly affected by “T_2 shine through” effects.

Diagnostic value of DW-MRI for “therapeutic time window”

When the concept of the ischemic penumbra was originally proposed, many studies were conducted to find the best “therapeutic time window” in clinical practice. Due to the hypoxic tolerance of brain cells, metabolic plasticity of the cerebral circulation, regional and chronological characteristics of cerebral hypoxia lesions, and different drugs and treatments, for example, individual cases exhibit differences in their respective “therapeutic time windows”[20-24]. The prevailing theory is that the “therapeutic time window” includes the time period up to 6 hours after the occurrence of cerebral infarction[25-26]. Nevertheless, this time frame remains controversial[6, 21, 27-28], as the ischemic penumbra tissue has been suggested to represent a dynamic process that is either completely present or completely absent, rather than existing for a fixed time period. A small number of patients have benefited from treatment 6 hours after onset, therefore demonstrating that a rigid “therapeutic time window” may not fit the true clinical situation.

In the present study, we examined data from 86 patients with cerebral infarction, and found that 19.6% (10/51) of the early cerebral infarction patients (detected on MRI) had an onset time of more than 6 hours, while 22.9% (8/35) of the middle and late infarction patients (detected on MRI) had an onset time of less than 6 hours. These data demonstrate that if a 6 hour “therapeutic time window” was chosen, then some early cerebral infarction patients (existing ischemic penumbra) would be excluded, while some middle and late cerebral infarction patients (no ischemic penumbra) would be included. Thus, we propose that the integrated images of a high DWI signal, a low ADC signal, and negative T_2WI and T_2FLAIR images should be utilized to determine the true “therapeutic imaging window”, which should aid in the accurate and timely clinical diagnosis and therapy

accurately for cerebral infarction. Furthermore, the use of a set 6 hours after infarction as the “therapeutic time window” requires more validation.

MATERIALS/SUBJECTS AND METHODS

Animal experiments

Design

A controlled, repeated-measures, randomized animal study.

Time and setting

Experiments were performed in the West China Medical Center, Sichuan University, China from May 2010 to December 2011.

Materials

A total of 78 healthy Wistar rats, aged 3 months old, weighing 300–350 g, of either gender, were purchased from the Animal Laboratory, West China Medical Center, Sichuan University, China (license No. SCXK (Chuan) 2008-24). Rats were housed in clean cages at 23 ± 1°C and under a 12-hour light-dark cycle. All rats were fasted for 24 hours before surgery, and experimental protocols were conducted in strict accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China[29].

Methods

Establishment of the middle cerebral artery occlusion animal model and grouping: Food was withheld from the rats for 24 hours prior to surgery. The modified suture-occluded method[5] was used to establish the middle cerebral artery occlusion model in the occlusion group. At each occlusion time point, we pulled an external light nylon fishing line until we felt a mild resistance. Reperfusion was performed for 1 hour in the reperfusion group. The control group received the same procedures as occlusion animals, except for carotid artery thrombosis.

MRI scan: All rats were scanned on a Signa HDx 3.0 T MR scanner (General Electric, Milwaukee, WI, USA) with a rat-specific phased array coil (5 cm in diameter) (Shanghai Chengguang Medical Technology Co., Ltd., Shanghai, China). Animals were placed in a supine position and subjected to coronal T_1WI scanning, taking the optic chiasm as the center line with a slice thickness of 2 mm, spacing 0 mm, field of view 4 cm × 4 cm, and matrix 128 × 128. DW-MRI parameters included an echo planar imaging sequence, repetition time of 9 000 ms, echo time of 102 ms, *b* value of 0 s/mm^2 and 1 000 s/mm^2.

The following formulas were used for analysis:
ADC = ln $(S_1/S_0)/(b_0–b_1)$[8], where S_1 is the signal intensity of b = 1 000 s/mm^2, S_0 is the signal intensity of b = 0 s/mm^2, and ln is the natural logarithm.
ΔDWI = (infarct area/total area of ipsilateral hemisphere) × 100%.
ΔADC = (infarct ADC/contralateral mirror area ADC) × 100%.

For the T_2WI, the following specifications were used: repetition time 2 000 ms, echo time 80 ms, slice thickness 2 mm, spacing 0 mm, field of view 4 cm × 4 cm, and matrix 128 × 128. For the T_2FLAIR, the following specifications were used: repetition time 10 000 ms, echo time 100 ms, thickness 2 mm, spacing 0 mm, field of view 4 cm × 4 cm, and matrix 128 × 128.

Pathological observation: After the MRI scan, rats were sacrificed with an overdose of 1% (v/v) pentobarbital sodium (30 mg/kg). The brains were fixed with 4% (w/v) paraformaldehyde using cardiac perfusion. The brains were removed by craniotomy for pathological observation. The brain tissues containing the right basal ganglia (infarct site) were embedded in paraffin, sliced into 6 μm thick sections, routinely stained with hematoxylin and eosin, and photographed under the optical microscope (Olympus, Tokyo, Japan). After conventional preparation and double staining using lead and uranium, the samples were observed and photographed under a transmission electron microscope (PECNAL G2F20, New York, NY, USA).

Statistical analysis: All measurements were expressed as mean ± SD and were statistically processed using SPSS 13.0 software (SPSS, Chicago, IL, USA). Multivariate repeated measures analysis of variance was performed to compare differences among various time points in the three groups. Paired *t*-test was used for the comparison between the occlusion and reperfusion groups. A value of $P < 0.05$ was considered statistically significant.

Clinical experiments

Design

A clinical retrospective study.

Time and setting

The experiments were performed in Affiliated Haikou Hospital of Xiangya School of Medicine, Central South University in China from March 2010 to April 2012.

Subjects

The clinical test included 86 patients, each of whom had been diagnosed with cerebral infarction. There were 52 males and 34 females in the group, and patient ages ranged from 46–83 years. There were 58 cases that exhibited typical partial symptoms of infarction. Limb weakness and speech problems were observed in 20 cases. Coma was present in four cases, and no obvious symptoms were shown in four cases. Within the group, a total of 92 cerebral infarction lesions were observed (with the exception of lacunar infarction). Of these cerebral infarction lesions, 56 were located in the basal ganglia, 18 in the brain stem, 10 in the temporal lobe, and eight in the frontal lobe.

Methods

All patients were scanned on a Signa HDx 3.0 T MR scanner (General Electric). T_2WI, T_2FLAIR, and DW-MRI scanning *via* fast recovery fast-echo sequence on cross-sections was used. The general parameters were as follows: 5 mm slice thickness and 1.5 mm spacing. For T_2WI: 6 000 ms repetition time and 113 ms echo time; and for T_2FLAIR: 9 000 ms repetition time and 165 ms echo time. For DW-MRI, using the echo-planar imaging sequence, the following parameters were used: 24 cm × 18 cm field of view, 128 × 128 matrix, selecting *b* values of 0 s/mm^2, 800 s/mm^2 for imaging, 6 000 ms repetition time, and 90 ms echo time. Each group was scanned using the above three sequence types. All patients had intact T_2WI, T_2FLAIR, DW-MRI, and ADC map information. Furthermore, the exact onset time of each patient was collected. After these treatments, all patients had an MRI review. The MRI data of the clinical cases were classified according to the results of the animal experiments.

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Conflicts of interest: None declared.

Ethical approval: All experimental protocols regarding the use of animals in the study were approved by the Institutional Animal Care and Use Committee of West China Medical Center, Sichuan University, China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/ patent application disputations.

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