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Original Article

Rat model of focal cerebral ischemia in the dominant hemisphere

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Abstract: In the human brain, the dominant hemisphere is more complex than the non-dominant hemisphere. Hence, cerebral ischemia of the dominant hemisphere often leads to serious consequences. This study aims to establish a rodent model of focal cerebral ischemia in the dominant hemisphere. The quadruped feeding test was used to screen 70 male Sprague Dawley rats. From this test, 48 rats with right paw preference were selected and randomly assigned numbers. Half were assigned to the dominant hemisphere ischemia (DHI) group, and the other half were assigned to the non-dominant hemisphere ischemia (NDHI) group. The middle cerebral artery was occluded 2 h before reperfusion. Neurological functions were tested. TTC and HE staining were performed. The volume of cerebral infarction was calculated. Rats in the DHI group had significantly worse neurological scores than rats in the NDHI group ($P < 0.05$). TTC staining indicated ischemia had more severe consequences in the dominant hemisphere than in the non-dominant hemisphere. The dominant hippocampus indicated severe neuronal loss and disorderly cellular arrangement. The volume of cerebral infarction was also greater in the DHI group compared to the NDHI group ($P < 0.05$). Compared to MCA occlusion in the non-dominant hemisphere, MCA occlusion in the dominant hemisphere caused greater impairment in neurological functions. The proposed rodent model is reliable and has high levels of reproducibility. Therefore, his model can be reliably for investigating the mechanism of focal cerebral ischemia in the dominant hemisphere of human brains.

Keywords: Dominant brain hemisphere, focal cerebral ischemia, animal model, rat

Introduction

Ischemia in the dominant hemisphere of the human brain leads to the loss of linguistic and other functional capabilities, and is one of the most severe types of clinical cases [1-3]. However, the lack of an ideal animal model has limited research in this area. Currently, the rat middle cerebral artery occlusion (MCAO) model is predominantly used for modeling cerebral ischemia in humans. Most researchers believe that there are no differences between the 2 hemispheres of the rat cerebrum. Therefore, they have randomly selected either left or right MCAO, which has led to significant differences in the resulting volumes of cerebral infarction [4]. The results of these experiments cannot be used to objectively analyze the mechanism of ischemia in the dominant hemisphere. In fact, the 2 hemispheres of the rat cerebrum are not entirely identical, with 1 hemisphere being dominant [5-8]. To address the lack of ideal animal models, this study aimed to establish a rodent model of focal ischemia in the dominant hemisphere.

Materials and methods

Experimental animals and grouping

The Ethics Committee of the People's Hospital of Ningxia Hui Autonomous Region approved this study. The dominant side of the rats was determined using the earliest quadruped rodent feeding test, as previously described [9] (**Figure 1**). Seventy Sprague-Dawley (SD) rats, weighing approximately 260-280 g, were allowed to acclimate to their environment for 7 d and were then fasted for 2 d. The rats were placed in individual cages, each with a small 1 × 1-cm opening in front that was just large enough for the rat's paw to pass though, but not its snout. The number of times that the left or right paw was used to reach for food was recorded and the probability for each paw was calculated.

Figure 1. Quadruped rodent feeding test to determine the rats' dominant side.

Based on the test results, the rats were categorized as exhibiting "right paw preference", "left paw preference", or "ambidexterity". The probability of the rats using their right, left, or both paws to reach for food was ≥ 29, ≤ 21, and 22-28, respectively. Next, 48 rats with right paw preference were selected, randomly labeled in numerical order, and then equally divided into 2 main groups: non-dominant hemisphere ischemia NDHI (rat nos. 1-24) and dominant hemisphere ischemia (DHI) (rat nos. 25-48). Within each group, a sham-operated sub-group acted as a control: rat nos. 18-24 and 43-48 from the NDHI and DHI groups, respectively.

Details on the various groups are as follows: (i) NDHI ischemia group: total n = 17; H&E staining, n = 9; TTC staining after 2 h ischemia, n = 5; 72 h reperfusion, n = 3; (ii) DHI ischemia group: Same as (i); (iii) sham-operated subgroup 1: total n = 7; H&E staining, n = 4; TTC staining, n = 3; and (iv) sham-operated subgroup 2: Same as (iii).

Rodent model of focal cerebral ischemia

We used the methods described in the study by Longa *et al*. [10]. The 48 rats were fasted overnight, were anesthetized with 8% chloral hydrate [(300 mg/kg, intraperitoneally (*i.p.*)], and fixed in a supine position. An incision was made on the paramedian region on the neck to expose the common, internal, and external carotid arteries. For the NDHI and DHI groups, the right or left common carotid artery was selected, respectively. The external carotid artery was then ligated.

The tip of a 4-0 monofilament surgical nylon strand was burnt in a windless environment using a mosquito coil to form a blunt end, which was then coated with poly-lysine. It was inserted through the external carotid artery 18-20 mm until mild resistance was encountered. The artery was blocked for 2 h before the strand was removed. The rats in the 2 sham-operated sub-groups went through the same procedure, except that their MCAs were not blocked. The rectal temperature of the rats, which was maintained at approximately 37°C with an electric heating pad, was monitored throughout the experiment. After the operation, the rats were placed in a 25°C air-conditioned room to allow them to regain consciousness.

Evaluation of neurological functions and the extent of damage

We referred to the methods described in the study by Guan *et al*. [11, 12]. The rats were evaluated before the operation and 24, 48, and 72 h after reperfusion. The areas assessed and scored covered areas for the state of consciousness, mobility, and sensory abilities. We have elaborated on the details of the tests below.

Tail suspension test (TST)

A score of "0" was given if there was no neurological injury. A score of "1" indicated the rat could not stretch its contralateral forepaw completely during the TST. A score of "2" indicated decreased ability to resist force on the contralateral forepaw, whereas "3" indicated that the paw was rotated toward the contralateral side.

Maintenance of posture test

This test refers to the duration that the rat was able to maintain its upper body posture when its tail was lifted. Rats were categorized according to the time needed to re-straighten their paws when either their contralateral forepaws or hind paws pointed towards their body. The scores were "0" (< 1 s), "1" (< 5 s), or "2" (> 5 s).

Horizontal righting reflex test

The rats were pushed towards their contralateral side to assess their reaction. If they resisted the contralateral force, a score of "0" was given. A score of "1" indicated a mild decrease in resistance initially, followed by subsequent recovery. If there was reduced resistance, the score was "2". If the rat fell towards its contralateral side, the score was "3".

Circling test

If the rat's movement remained in straight lines, a score of "0" was given. Movement towards the right scored a "1", whereas a circling movement was a "2". If there were no movement at all, the score was "3".

The total score for the above tests ranged from 0-11 points, with "0" representing normal behavior and "11" indicating severely impaired neurological functions. After these tests were conducted, different procedures were performed on different rats in accordance with the experimental groupings described in Section 2.1.

TTC staining

TTC is a lipid-soluble, light-sensitive compound that has been used since 1958 to detect tissue ischemia in mammals. It remains a commonly used indicator to evaluate ischemia within the brain. As a proton acceptor of pyridine nucleotide transhydrogenase in the respiratory chain, TTC undergoes dehydrogenation and turns red in normal tissues. However, it remains white in hypoxic tissue due to the lack of dehydrogenation resulting from the decrease in dehydrogenase activity.

The brains of the 16 rats identified for TTC staining were sliced transversely into 5-6 slices using a silicon steel microtome with Plexiglass 2-mm tissue matrix. The first cut was made at the midpoint between the frontal pole and optic chiasm, the second at the optic chiasm, the third at the infundibular stalk, and the fourth between the infundibular stalk and the posterior pituitary. The brain slices were placed in 2% TTC, covered with aluminum foil to shield them from light, placed in an incubator maintained at 37°C for 15-30 min, and swirled around to stain the brain slices evenly. The slices were scanned and analyzed to determine the total area of cerebral ischemia.

H&E staining

Routine deparaffinization, staining, dehydration, and clearing were performed. The 26 samples reserved for H&E staining were mounted on neutral resin and observed using light microscopy.

Volume of cerebral infarction

After 72 h of reperfusion and anesthesia, the brains of the 6 rats were collected, perfused, and fixed for 2 h in a mixture of 20% sucrose and 4% paraformaldehyde. They were then transferred to 0.01 M PBS with 30% sucrose solution until they sank. The samples were frozen and sliced into coronal sections, each approximately 30-μm thick. One slice was extracted from every 10 slices, placed in cytoprotective solution, and stored at -20°C.

For H&E staining, a Leica Q500 IW Imaging Workstation (Leica, Bensheim, Germany) was used to scan the sections, and data were entered into a computer for processing. The formula used to calculate the volume of cerebral infarction was:

$$V = t\,(A1 + A2 + \ldots + An) - t\,(A1 + An)/2$$

Where V is the volume of infarction (mm^3), t is 30 μm (the distance between adjacent slices), and A is the area of infarction on each slide.

Statistical analyses

All data are presented as the mean ± standard deviation ($\bar{x} \pm S$). The Statistical Package for the Social Sciences (SPSS) version 11.5 was used to process the data. F-max tests were performed on the data to test for homogeneity of variance. If the variances were homogeneous, one-way analysis of variance (ANOVA) was performed for inter-group comparisons. If the vari-

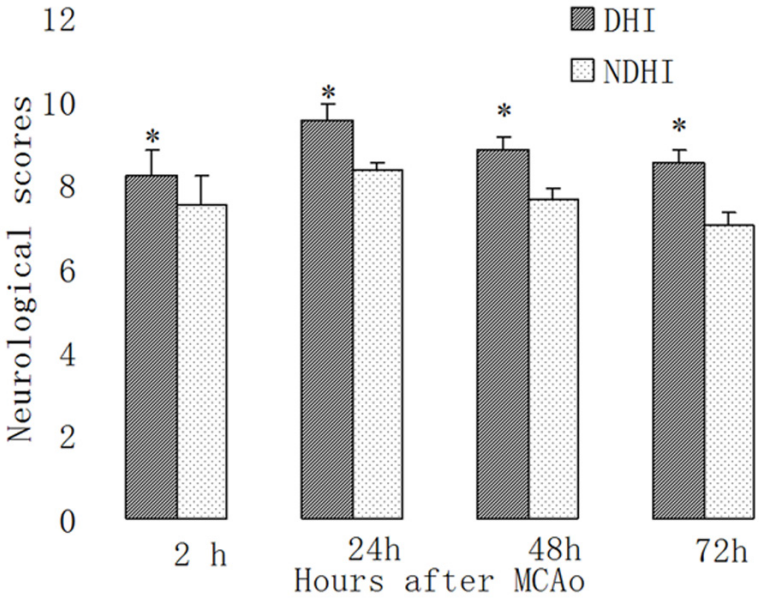

Figure 2. Scores for impairment in neurological functions. NDHI: Non-dominant hemisphere ischemia group; DHI: Dominant hemisphere ischemia group, *P < 0.05.

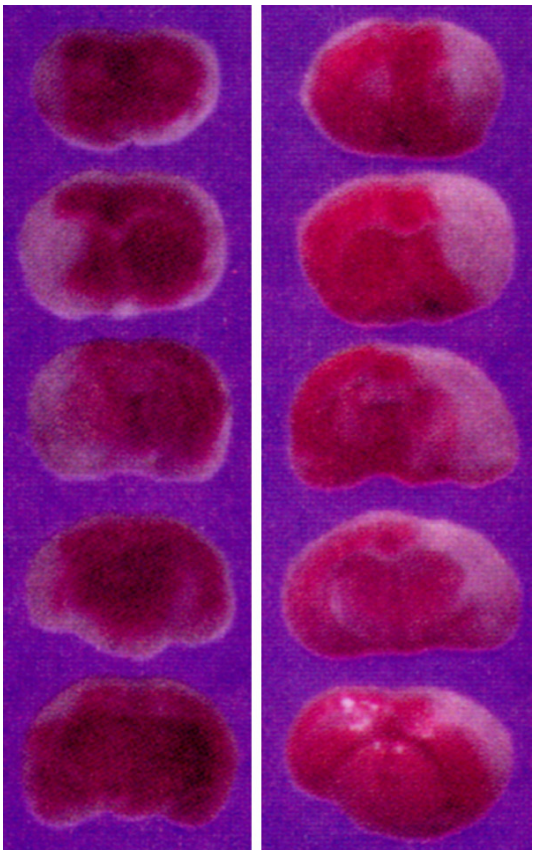

Figure 3. TTC staining of rat brain tissue slices (TTC 2 × magnification). Left: NDHI group; Right: DHI group.

ances were non-homogeneous, non-parametric statistics were used. The results indicated that the differences were significant ($P < 0.05$).

Results

Score comparisons for impairments in neurological functions

All rats that underwent MCA occlusion showed a certain extent of impairment in neurological functions. The DHI group had significantly worse scores than the NDHI group for the tests conducted at 24, 48, and 72 h after reperfusion. For the 4 tests (tail suspension, maintenance of posture, horizontal righting reflex, and circling), the scores for the DHI and NDHI groups were 8.3 ± 0.5 *vs.* 7.6 ± 0.4, 9.4 ± 0.3 *vs.* 8.6 ± 0.1, 8.9 ± 0.4 *vs.* 7.7 ± 0.1, and 8.6 ± 0.2 *vs.* 7.1 ± 0.2, respectively (**Figure 2**). These differences were statistically significant ($P < 0.05$) and indicate that focal ischemia in the dominant hemisphere causes more severe and lasting impairments in neurological functions.

TTC staining

All normal brain specimens were stained deep red but the infarcted areas of brain specimens that had undergone MCAO remained white (**Figure 3**). Infarction was consistently observed in the brain specimens of rats that had undergone 2 h ischemia and 72 h reperfusion. Processing using an image analyzer revealed that the volume of infarction was greater in the DHI group than in the NDHI group. This indicates that the impairment arising from focal ischemia was more severe in the dominant hemisphere than in the non-dominant one.

Comparison of cerebral infarction volumes

Observation of H&E staining using light microscopy revealed that infarction was clearly observed in the cerebral cortex, putamen, and caudate nucleus. There was dilation of Virchow-Robin spaces (VRS), decreased number of neu-

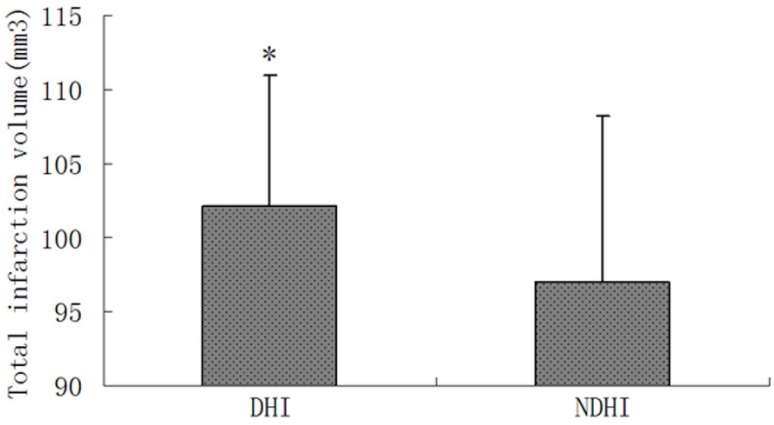

Figure 4. Comparison of cerebral infarction volume (mm^3) after focal cerebral ischemia. NDHI: Non-dominant hemisphere ischemia group; DHI: Dominant hemisphere ischemia group, *P < 0.05.

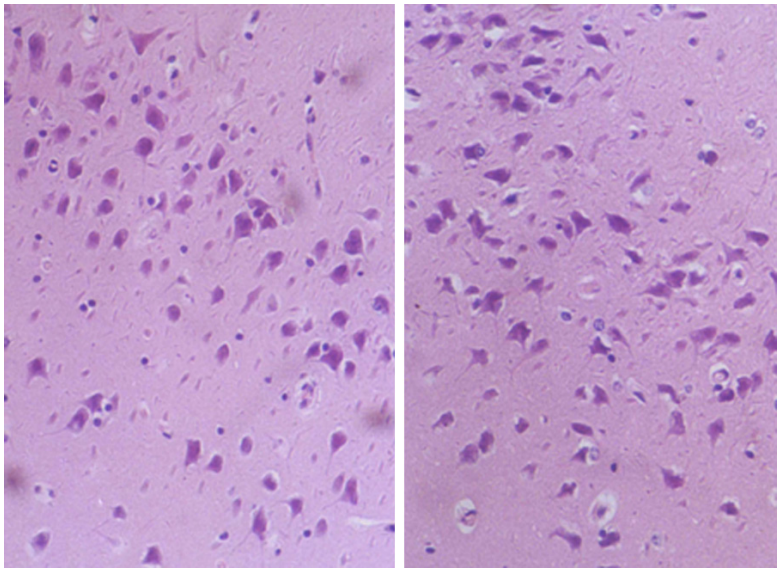

Figure 5. H&E staining indicating changes in hippocampal structure after focal cerebral ischemia (H&E 400 × magnification). Left: NDHI group; Right: DHI group.

rons, cellular edema, and partial karyopyknosis. After processing using the image analyzer, it was found that the volume of cerebral infarction was smaller in the NDHI group than in the DHI group (**Figure 4**). The differences were statistically significant (P = 0.04). These results indicate that the volume of cerebral infarction was larger in the dominant hemisphere compared to the non-dominant one.

Dominant hippocampal H&E staining

Seventy-two hours after focal ischemia in the dominant hemisphere, there was coagulative necrosis in the ischemic center, unclear neural cytoplasm and nuclei structure, strong eosinophilic staining, infiltration of polymorphonuclear leukocytes in the area surrounding the necrotic center, leukoaraiosis, the formation of large numbers of vacuoles, a severe decrease in the number of neurons, and disorderly arrangement of hippocampal cells (**Figure 5**). Coagulative necrosis was also observed in the ischemic center of the non-dominant hemisphere, albeit to a lesser extent. Neuronal degeneration surrounding the necrotic foci was reduced, perivascular edema was milder, changes to the interstitial space were also relatively smaller, and surviving normal neurons were found among the degenerated neurons within the hippocampus.

Discussion

There are obvious differences in the flexibility and habitual usage between the 2 human hands (i.e., laterality). This is generally presented as differences in preference, adroitness, and skillfulness. There is a clear dominance (90%) of right-handedness in the normal population. For the vast majority of normal right-handed, and a few left-handed people, the language center is located within the left cerebral hemisphere. It is located in the right cerebral hemisphere for most left-handed people.

Compared to the non-dominant hemisphere, the dominant hemisphere of the human brain has more numerous and complex functions. The right and left hemispheres play different roles in behavior and higher order mental activities. Modern neurophysiological studies have concluded that the left cerebral hemisphere plays a decisive role in linguistic functions, logical thinking, analytical skills, use of skills, and

computation. The right hemisphere has a significant role in spatial abilities, shape recognition, music, art, comprehensive ability, and transient visual memory. Ischemia of the dominant hemisphere in the human brain resulting in the loss of linguistic and other functional capabilities is one of the most severe types of clinical conditions. However, research in this area is sparse, which is likely due to the lack of ideal animal models.

Due to the limitations in research conditions, the current studies to understand human focal cerebral ischemia commonly employ MCAO in rats, the process of which quite closely resembles that in humans. However, results regarding the size and distribution of infarction volume caused by MCAO in rats vary between reports. Differing results from different experiments make statistical analyses difficult. In addition to the differences in the rat species used and occlusion depth in different experiments, another significant factor is the random occlusion of the left or right MCA in the rats. Previously, there was no proper basis for the selection of which MCA to occlude, and the decision was solely based on the personal habits of the researchers.

In fact, paw movements in rats are similar to hand movements in humans. Laterality in the brain is not unique to humans and is common in the animal kingdom [9]. Unilateral dominance is similarly observed for rat paws, which is determined by the dominant hemisphere in the rat's brain [11-14]. Robinson *et al*. [15] reported that the right MCAO in SD rats resulted in hyperactivity. This phenomenon lasted 2-3 weeks, with norepinephrine levels in the ipsilateral and contralateral cerebral cortices as well as the locus coeruleus decreasing by 30%. In addition, the dopamine concentration in the substantia nigra was reduced by 20%. However, hyperactivity was not observed for left MCAO, and there were no changes in catecholamine levels. Therefore, these researchers speculated that the different behavioral and biochemical changes induced by ischemia in the right and left hemispheres were due to asymmetry in rat brain anatomy and physiological functions, namely hemispheric laterality.

Cechetto *et al*. [16] observed that for right MCAO in rats, there might be a sudden occurrence of cardiovascular system diseases and a peak in amygdala dynorphin level after 3-5 d. These have similar temporal patterns as stress-induced cardiovascular responses, which may be related to cortical damage in the right hemisphere.

A series of local and international studies have been conducted on the MCA because it is susceptible to cerebrovascular disease. When MCAO was first proposed by Bannister *et al*. [17], the right MCA was occluded. Subsequently, Longa *et al*. [10] and Kuge *et al*. [18], who specialized in animal models, also reported their findings based on right MCAO. Left MCAO has been performed in fewer studies, although the reason for this is unexplained.

Some damage to the vagus nerve is inevitable when producing rat MCAO through neck surgery. The left vagus nerve regulates and directly impacts the heart and can cause fluctuations in blood pressure. We have often observed in our experiments that the separation of the vagus nerve climbing fibers in the carotid artery and separation of the internal carotid artery caused the rats to struggle, cough up phlegm, and breathe irregularly. Given the similarities between human and rat brains from a structural and functional point of view, attention should be paid to the asymmetry of brain structure when selecting a side for the MCAO model. The impact of other influencing factors should be minimized by maintaining regular breathing and stable blood pressure. The side of the MCA chosen for occlusion should not be determined randomly, but rather chosen in accordance with the purpose of the study.

In previous studies, adoption of the MCAO method was influenced by various factors including rat strain, batch and body mass, as well as the operator's proficiency. Hence, vascular occlusion could not be fully determined. In this study, the various research conditions were strictly controlled. This included using only SD rats and improving the preparation of the suture and surgical procedures. We repeatedly tried various ischemia parameters, standardized the embolic strand texture and treatment of the tip, avoided excessively severe cerebral ischemia or vascular injury (which might lead to the death of the rats), and standardized and simplified the entire surgical process.

Locally, the Bederson scoring system is frequently used to assess neurological deficits

due to the simplicity of the system. However, this method has its limitations because it is not able to capture feelings or the overall physiological state. The Garcia and Clark scoring systems are more comprehensive and have high levels of operability. Numerous neurological functions are evaluated, hence their wide international usage. Comprehensive evaluations of motor and sensory functions as well as voluntary movement have led to a high degree of correlation between the scores and the identification of cerebral lesions. The Garcia scoring system should be used whenever possible in evaluating neurological function in focal cerebral ischemia [11, 12]. In this study, an improved evaluation of neurological deficits, which was comprehensive and easy to implement, was adopted.

In summary, a behavioral test was first performed to determine the dominant side of the rats, and then the brains of the rats were operated to create a focal cerebral ischemia model. Neurological impairment scores, TTC staining, H&E staining, and infarction volume were used to confirm that cerebral ischemia in the dominant hemisphere caused more severe impairment and damage. Here, this rodent MCAO model can be replicated consistently, thus providing a reliable tool for investigating the mechanism of focal cerebral ischemia occurring in the dominant hemisphere of human brains.

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Disclosure of conflict of interest

None.

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