Neurological Dysfunctions Versus Regional Infarction Volume After Focal Ischemia in Mongolian Gerbils

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Background and Purpose—With advances in the therapy of stroke at the postacute phase, the use of animal models for chronological and region-specific evaluation of neurological function has become increasingly important. Our aim was to test long-term behavioral dysfunction in gerbils after focal ischemia and to correlate the results with the regional distribution of infarction in the coordinating cortical regions.

Methods—Repetitive unilateral hemispheric ischemia (two 10-minute occlusions, 5-hour interval) was induced in Mongolian gerbils. The elevated body swing test (EBST), bilateral asymmetry test (BAT), and T-maze test were performed to assess asymmetrical motor behavior, somatosensory deficit, and spatial cognitive dysfunction during 4 weeks after ischemia. The results were correlated against the regional infarction volume of the primary motor, somatosensory, and primary visual cortices at 4 weeks after ischemia.

Results—In all postischemic gerbils, persistent sensorimotor and cognitive dysfunctions were detectable throughout the postischemic period. Histological examination revealed that a cortical zone of infarction surrounded the selective neuronal death in the ipsilateral cerebral hemisphere. The regional infarction volumes of the primary motor, somatosensory, and visual cortices were significantly correlated with the scores of the EBST, BAT, and T-maze test, respectively. These combinations had the highest regression coefficient of all pairs.

Conclusions—Postischemic motor and somatosensory functions were significantly correlated with regional infarction volumes in the corresponding cortical regions. In gerbils, visual abnormality could be independently detected by the T-maze test. Such regional analyses of ischemic lesions would be useful for investigating the functional outcomes of stroke therapy. (Stroke. 2003;34:1501-1506.)

Key Words: behavior, animal / cognition / stroke, experimental / vision disorders / gerbils

Middle cerebral artery occlusion in rodents, representing the most prevalent form of stroke in humans, is most commonly used for experimental cerebral ischemia. Various neurological symptoms develop after middle cerebral artery occlusion, and it is therefore useful for assessment of potential therapeutic treatment for human stroke. However, it has been shown in middle cerebral artery occlusion models that the intensity of the neurological deficit is not significantly correlated with the total size of the infarction.2,3

The reason for the marginal correlation between neurological and morphological findings may be lack of region-specific analyses. It has been shown that cerebral injury localized in the striatum, primary motor cortex, somatosensory cortex, or primary visual cortex induces region-specific neurological dysfunctions in rodents.5–7 Because brains develop widespread ischemic lesions after experimental focal cerebral ischemia, it is likely that researchers need region-specific analysis between neurological dysfunctions and tissue injury rather than analysis of correlation with total infarct size.

The aim of the present study was to elucidate the relationship between the intensity of ischemic tissue injury evaluated as regional infarction volume at several regions of interest (ROIs) and the severity of region-specific neurological dysfunctions. We used a gerbil model of focal ischemia, in which the common carotid artery is occluded unilaterally twice for 10 minutes with a 5-hour interval between the ischemic insults. This model is known to induce infarction mainly in the cerebral cortex,6,9 which is suitable for quantitative regional analysis of ischemic lesion.

Neurological examinations were performed with the use of the elevated body swing test (EBST), bilateral asymmetry test (BAT), and T-maze test to assess asymmetrical motor behavior, somatosensory deficit, and spatial cognitive dysfunction, respectively. Each behavioral test has been used generally for the evaluation of long-term neurological dysfunctions and...
therapeutic effects in experimental ischemia of rodents. Neurological symptoms are known to fluctuate at the acute stage in both clinical and experimental strokes. The process leading to infarction and selective neuronal death may take several days to weeks after an ischemic episode. We therefore examined chronological changes in neurological dysfunctions and correlated them with histological changes at 4 weeks after ischemia.

Materials and Methods

Animals and Experiments

The animal experiments were approved by the Animal Care and Use Committee of Tokyo Medical and Dental University. Eighteen Mongolian gerbils of both sexes between 16 and 20 weeks of age and weighing 60 to 72 g were used in this experiment. Animals, housed in groups of 3 or 4, were maintained on a 12-hour light/dark cycle with unlimited access to food and water.

Surgical Procedures

Animals were divided into 2 groups: carotid artery–occluded animals and sham-operated animals. For the induction of ischemia, the animals were anesthetized with 2% isoflurane, the right common carotid artery was occluded with a mini vascular clip, the clip was removed after 10 minutes of occlusion, and the animals were allowed to recover from anesthesia. During the initial carotid artery occlusion, stroke symptoms were evaluated according to a stroke index (SI) of 0 to 25 points, as reported previously. Animals manifesting an SI score of >10 were selected as “postischemic animals.” In postischemic animals, the second 10-minute period of ischemia was induced similarly 5 hours later. Sham-operated animals were operated in the same manner except for the right carotid artery occlusion.

Behavioral Tests

Postischemic animals and sham-operated animals were subjected to a series of behavioral tests during the 28 days after stroke had been induced. The EBST, BAT, and T-maze test were conducted before surgery and on days 3, 4, 7, 14, 21, and 28 after stroke. The researcher conducting the behavioral testing and scoring was blind to the experimental condition.

Elevated Body Swing Test

The EBST was used to evaluate asymmetrical motor behavior. Animals were held by the base of the tail and elevated approximately 10 cm above a tabletop. The direction of body swing, defined as an upper body turn of >10 degrees to either side, was recorded for 1 minute during each of 3 trials per day. The numbers of left and right turns were counted, and the percentage of turns made to the side contralateral to the stroked hemisphere (percent left-biased swing) was determined.

Bilateral Asymmetry Test

The BAT is a test of unilateral somatosensory dysfunction. Two small adhesive-backed paper dots (each 60 mm²) were used as tactile stimuli on the distal-radial region of the wrist of each forelimb. Animals were observed in a cage (20×40×30 cm). The time, to a maximum of 3 minutes, that it took each gerbil to remove each stimulus from the forelimb (removal time) was recorded in 3 trials per day. Individual trials were separated by at least 3 minutes.

T-Maze Test

The T-maze spontaneous alternation task has been reported to test exploratory behavior and working memory. In a lighted room, the animals were allowed to alternate between the left and right goal arms of a T-shaped maze, which measured 60 (stem)×25 (arm)×10 (width) cm, throughout a 15-trial continuous alternation session. Once they had entered a particular goal arm, a guillotine door was lowered to block entry to the opposite arm. The door was removed only after the animals returned to the start arm, thus allowing a new alternation trial to be started. Their behavior was not reinforced by punishment or reward and was traced with a video-tracking system (PanLab). The position of each gerbil in each arm and stem and the entered arm were analyzed with the use of Smart software (PanLab). After we had tracked the movement of each animal in the T-maze, the spontaneous alternation rate was calculated as the ratio of the alternating choices to the total number of choices (50%, random choice; 100%, alternation at every trial; 0%, no alternation). We also calculated the percentage of choices of the goal arm ipsilateral to the stroked hemisphere (percent right-biased rate). Furthermore, T-maze tests were conducted in a completely darkened room to investigate the behavior of the gerbils in the absence of visual input.

Histological Analysis

At the end of the observation period, animals were anesthetized deeply with diethyl ether and killed with perfusion of 4% paraformaldehyde. The brain was cut into 6 serial coronal sections every 2.0 mm from the level of the anterior pole of the caudate nucleus to the posterior pole. The sections were immersion-fixed in buffered formalin and embedded in paraffin. Each coronal section was sliced in thickness of 6 μm and stained with Klüver-Barrera. Areas of infarction, ipsilateral hemisphere, and contralateral hemisphere in 6 coronal sections were traced on an image of each scanned section with the use of National Institutes of Health image analysis software. Total infarct volume (indirect lesion volume) of the ipsilateral hemisphere was calculated as a percentage of the volume of the contralateral hemisphere, as previously reported. We calculated the regional infarction volume in each ROI in the primary motor, somatosensory (forelimb area), and primary visual cortices according to previous reports.

Statistical Analysis

The data were analyzed by repeated-measures ANOVA with independent variables of treatment group and day of testing. An unpaired t test was used for statistical comparison between groups for each measurement day. The level of statistical significance was set at P<0.05. All values are presented as mean±SD.

For all postischemic animals, correlation analyses were performed between measures of each quantitative ischemic lesion and average behavioral measures. Measures of ischemic lesions included total infarction volume and the regional infarction volume in each ROI.

Results

Postischemic animals (n=8) with SI score of >10 and sham-operated animals (n=10) were selected for behavioral tests and histological analysis.

Elevated Body Swing Test

The percent left-biased swing was significantly higher in postischemic animals than in sham-operated controls throughout the 4-week postischemic period (Figure 2A). The percent left-biased swing was 52±5.3% before occlusion and increased at 77.2±6.6% on day 4 and 70.8±4.8% on day 28. Postischemic animals showed a strong and persistent ten-
dency to turn their upper bodies to the side contralateral to the stroked hemisphere, whereas sham-operated controls showed no bias ($P<0.001$). In addition to the biased body swing, all postischemic animals showed flexion of the forelimb contralateral to the stroked hemisphere while they were suspended by the tail.14

**Bilateral Asymmetry Test**

The removal time from the contralateral forelimb was significantly longer for postischemic animals than for sham-operated controls throughout the 4 weeks (Figure 2B). The removal time was $19 \pm 9.3$ seconds before occlusion and was prolonged to $148 \pm 21$ seconds on day 4 and $88 \pm 19$ seconds on day 28 after ischemia. Postischemic animals took longer to remove a contralateral tape than an ipsilateral tape ($P<0.001$). Sham-operated controls showed no difference at any measurement day.

**T-Maze Test**

Before occlusion, animals tended to choose a goal arm alternately and to choose left and right with equal frequency. Sham-operated controls continued to show no difference after surgery. In the T-maze, both sham-operated and postischemic animals moved straight in the middle of the arm and stem (Figure 3A and 3B), and the rotational behavior was not observed. The spontaneous alternation rate was below the random alternation rate (<50%) in all the postischemic animals (Figure 2C). The rate was $75.4 \pm 5.6\%$ before occlusion and was significantly reduced to $23.2 \pm 11.4\%$ on day 4 and $38.6 \pm 8.5\%$ on day 28. Animals tended to choose only the right goal arm after ischemia. The percent right-biased rate was $49.4 \pm 4.8\%$ before occlusion, $77.3 \pm 7.2\%$ on day 4, and $67.9 \pm 8.8\%$ on day 28 after ischemia, which was significantly higher than the rate in the sham-operated controls (49.4±5.2%) (Figure 2D). In the darkened room, the choice bias of postischemic animals was significantly less apparent than in the lighted room, resulting in a percent right-biased rate of $62.3 \pm 6.8\%$ in the darkened room compared with $74.8 \pm 5.9\%$ in the lighted room ($P<0.05$) (Figure 3C).
Histology

Sham-operated controls showed no ischemic changes. In postischemic animals, infarction was confined to the ipsilateral cerebral cortex, hippocampus, dorsolateral nucleus of thalamus, and caudate nucleus (Figure 4). In the rostral cortex, the infarction involved the entire cortical layer, and in the caudal cortex the infarction was laminar and confined to the middle cortical layer. Surrounding the infarction, a zone of selective neuronal death was observed. The average total infarct volume was 31.2±8.7% of the cerebral hemisphere. In postischemic animals, the regional infarction volume of ROIs in the primary motor, somatosensory (forelimb area), and primary visual cortices was 39.9±9.7%, 44.8±11.9%, and 16.2±4.3%, respectively (Figure 4C).

Correlation Analysis

In postischemic animals, ischemic lesion measurements were correlated against behavioral test results. A marginal correlation with total infarct volume was found for spontaneous alternation rate \((r=0.725, P<0.05; \text{Table})\). However, correlations with total infarct volume were not found for percent left-biased swing, removal time, or percent right-biased rate.

The regional infarction volume in the primary motor, somatosensory, and primary visual cortices was significantly correlated with percent left-biased swing \((r=0.886, P<0.01)\), removal time \((r=0.852, P<0.01)\), and spontaneous alternation rate \((r=-0.865, P<0.01)\), respectively (Table and Figure 5). The regional infarction volume in the primary visual cortex was also significantly correlated with percent right-biased rate \((r=0.849, P<0.01)\). Correlations in the other combinations between regional infarction volume and behavioral test results were weaker (Table).

Discussion

Repetitive Ischemia

We used a gerbil model of repeated unilateral carotid artery occlusion.\(^8,9\) This model induces ischemic infarction, mainly in the cerebral cortex. The distributions of infarction and selective neuronal death in the present study (Figure 4) are similar to those in previous reports.\(^8,9\) This distribution in the cerebral cortex is suitable for use in analysis of the correlation between regional ischemic damage in the cortex and various neurological dysfunctions.
Behavioral Tests

We used 3 behavioral tests, the EBST, BAT, and T-maze test, which have been used to measure 3 different neurological dysfunctions: asymmetrical motor behavior, somatosensory deficit, and cognitive dysfunction, respectively. Measurable impairments were observed during 4 weeks of the postischemic period in all tasks.

We found significant correlations between the results of these behavioral tests and regional infarction volume in the cerebral cortex functionally corresponding to the tasks. In this model, however, ischemic tissue change is also found in the ipsilateral caudate nucleus, thalamus, and hippocampus, which might induce the observed sensorimotor and cognitive dysfunctions.

In an EBST, striatum-lesioned animals show asymmetrically biased swing. The direction of swing is ipsilateral to the lesioned side owing to the ipsilateral postural turning of the trunk.10 Cortex-lesioned animals, in contrast, show biased swing contralateral to the lesioned side and have a posture of contralateral forelimb flexion.2,24 The swing bias in the present study was contralateral to the lesioned side, and the contralateral forelimb retained a flexion posture. The biased body swing in our model was therefore probably induced by the cortical lesion.14,25 Forelimb somatosensory deficits were evaluated by the BAT. Somatosensory stimulations in rodents are projected from the ventrobasal complex of the thalamus to the primary somatosensory cortex.26 In our model, infarction was found in both the somatosensory cortex and the thalamus. However, this thalamic lesion did not involve the forelimb-innervation area of the ventrobasal complex.26 In addition, a lesion in the forelimb-inervated somatosensory cortex in rat induces contralateral forelimb somatosensory disturbance, as evaluated by the BAT.7 These results indicate strongly that the somatosensory deficits of the contralateral forelimb found in this study were induced by ischemic damage to the somatosensory cortex rather than to the thalamus.

Choice Bias in the T-Maze Test

The spontaneous alternation rate in the T-maze test has been reported to reflect exploratory behavior and spatial memory, which allow animals to acquire information about novel places and things.15 The hippocampus is widely recognized as playing a role in exploration of novelty and spatial memory.27,28 In animals with hippocampal injury, the spontaneous alternation rate is known to decrease to approximately 50% (random choice) but not below this level.15 In our previous experiment in a gerbil model in which we induced unilateral CA1 neuronal death of the hippocampus, we observed a decrease in the spontaneous alternation rate to approximately 50%,29 which is in accord with the results of previous reports on experimental hippocampal injury. However, the postischemic animals in our present study tended to choose the right goal arm of the T-maze, as shown by a marked decrease (below 25%) in the spontaneous alternation rate. We therefore need to consider that widespread ischemic lesions in other areas may be responsible for this decrease by inducing motor, somatosensory, or visual dysfunction.

Chopp et al25 reported that mice with focal ischemia showed a biased turn ipsilateral to the stroked side when they entered a V-shaped corner and their vibrissae on both sides touched the side walls of the corner. Their results indicate the influence of somatosensory dysfunction on choice bias in mice. However, the results of our gerbil experiment appear to indicate that the choice bias of these animals is related to visual abnormality. We observed postischemic animals in the T-maze moving straight in the middle of the stem and arms without touching the side walls with their vibrissae. When the postischemic animals were placed

| Correlation Between Neurological Symptoms and Ischemic Lesions in Postischemic Animals |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| EBST (%) (left-biased swing)    | BAT (removal time)               | T-maze (alternation rate)       | T-maze (% right-biased rate)    |
| Total infarct volume            | Total infarct volume             | Total infarct volume             | Total infarct volume             |
| r                               | P                               | r                               | P                               |
| 0.503                           | 0.442                           | -0.725                          | *                               |
| Primary motor cortex            | Somatosensory cortex            | Primary visual cortex            |somatosensory cortex            |
| 0.886                           | 0.882                           | 0.232                           | 0.865                           |
| **                             | **                              | **                              | **                              |

**P<0.01; *P<0.05 (regression analysis).
in a darkened room to minimize visual acquisition, they chose a goal arm contralateral to the stroked side (Figure 3C) more often than in the lighted room. No rotational behavior signifying asymmetrical motor deficit was detectable during the T-maze test. These results indicate that the decrease of choice bias in the gerbil in the absence of light is not caused by somatosensory dysfunction or motor deficit but by changes in visual input.

Mongolian gerbils have been reported to be visual animals and to show a greater variety of visual behaviors than rats or mice, which have poorly developed visual systems.3 In the gerbil, these visual behaviors, including visually guided head turning and jumping tasks or locomotor elicitation by visual targets, are markedly influenced by damage to the primary visual cortex.5,30–32 Our finding of the importance of visual influences in the T-maze agrees with the results of these studies. Our ischemic model would thus be suitable for the analysis of visual abnormalities related to ischemic damage in the primary visual cortex.

Correlation Between Regional Infarction Volume and Behavioral Outcome
The relationship between neurological dysfunction and distribution of ischemic lesions has been investigated in various ischemia models. In most studies, the severity of ischemic injury was quantified by measuring the total infarct volume.2,3 However, these reports often revealed marginal or no correlation between behavioral measures and ischemic lesion size. Similarly, we found only marginal correlation between total infarct volume and spontaneous alternation rate. No significant correlation was found in the other behavioral measures.

Thus, we anticipated that the EBST, BAT, and T-maze results would reflect the degree of ischemic tissue injury to the functionally corresponding brain structures. In fact, we found significant correlations between the results of the EBST, BAT, and T-maze test and regional infarction volume in the primary motor, somatosensory (forelimb area), and primary visual cortices, respectively.

There are an increasing number of nonlethal cases of stroke in the human population, and our experimental paradigm for independent evaluation of various neurological dysfunctions would be useful for therapeutic studies of such cases of cerebral ischemia.

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