

Involvement of Cortical Damage in the Ischemia/Reperfusion-Induced Memory Impairment of Wistar Rats

Jin Tae Hong^{1*}, Seung Rel Ryu¹, Hye Jin Kim¹, Sun Hee Lee¹, Byung Moo Lee², and Pu Young Kim¹

¹National Institute of Toxicological Research, Korea Food and Drug Administration 5, Nokbun-dong, Eunpyung-gu, Seoul, 122-704, Korea and ²Division of Toxicology, College of Pharmacy, SengKyunKwan University Chunchun-dong 300, Suwon, Kyunggido, 440-746, Korea

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The effect of ischemia/reperfusion-induced neuronal damage on the memory impairment were investigated using active avoidance and Morris water maze tasks in Wistar rats. Focal ischemia was induced by 1 h occlusion of the right middle cerebral artery (MCA) of Wistar male rats. Reperfusion was induced by releasing the occlusion and restoring the blood circulation for 24 h. The acquisition and preservation memory tested by active avoidance showed a significant difference between the sham and ischemia/reperfusion group. The water maze acquisition performance was also significant difference between sham and ischemia/reperfusion groups in both latency and moving distance. The infarction volume was increased by the ischemia/reperfusion. Furthermore, the cresyl violet staining of the ischemia/reperfusion brain showed severe neuronal damage (pyramidal cell loss) in the cortex in addition to the striatum lesion of brain. This study shows that pyramidal cell damage in the cortex lesion may be partially related to memorial disturbance in the ischemia/reperfusion brain injury.

Key words: Ischemia/reperfusion, Wistar rats, Behavioral deficits, Cortical damage

INTRODUCTION

Stroke is a common type of ischemia/reperfusion-mediated brain injury, and a major disease in central nervous system (Petito *et al.*, 1990; Pelsinelli, 1996). With a view toward improving treatment of stroke patients, not only the basic aspects of the disease but also clinical signs such as memory and learning abilities should be determined. A key factor in these processes is the development of an animal model that mimics closely the neuropathological consequences of stroke (Tamura *et al.*, 1981; Yamamoto *et al.*, 1988). While there have been already a significant number of animal models of stroke, suitable animal models to fit stroke patient clinically are being investigated. Animal stroke models must have a relatively constant of infarction with good reproducibility in inexpensive animals. In addition, quantitative association between brain injury and clinical outcomes are also required. To provide better means for early diagnosis and treatment that would improve the patient therapy, in the present study we

established an ischemia/reperfusion animal model using Wistar rats by the middle cerebral artery occlusion method. We then examined the effect of ischemia/reperfusion-induced neuronal damage (cortex region) on the memory impairment.

MATERIALS AND METHODS

Animals

Wistar rats were maintained in accordance with the National Institute of Toxicological Research of Korea Food and Drug Administration guideline for the care and use of laboratory animals. Rats were housed 4-5 per cage and maintained at $22 \pm 2^\circ\text{C}$ with a constant humidity at least for 1 week prior to the commencement of the experiments. All the animals were allowed free access to food and water before and after ischemia surgery.

Ischemia surgery

The acclimated Wistar rats were anesthetized with a gas mixture of 75% N₂O and 25% O₂. The middle cerebral artery was occluded using sugita aneurysm clips. During the occlusion period and postoperative period, the animals were kept on a thermostat-control warming plates in order

Correspondence to: Jin Tae Hong, National Institute of Toxicological Research, Korea Food and Drug Administration, 5, Nokbun-dong, Eunpyung-gu, Seoul, 122-704, Korea
E-mail: jinthong@kfda.go.kr

to maintain body temperature at 37°C to prevent hypothermia. Following the occlusion, the clips were removed to restore blood for recirculation. The same surgical operated animals without cerebral artery ligation were served as control animals.

Morphometric determination of infarct volume

The cross-sectional area of the infarction on the surfaces of each brain slice was defined by 2,3,5-triphenyltetrazolium chloride (TTC) staining for detection of ischemia infarction area of brain. After ischemia the rats were received intracardiac perfusion of 0.9% buffered saline. The brain was then removed, and cut into 2 mm serial slices starting 1 mm from the frontal pole. The coronal slices were then immersed in a 2% phosphate-buffered for 50 min at 37°C. After staining with TTC, slices were fixed in 10% phosphate-buffered formalin and the infarction area was determined by image analyzer with LeicaQwin program (Leica Microsystem Image Solution Ltd., Combrige, England). The infarct area (mm²) from each thicken-brain slice was determined, and then the infarct volume (mm³) was calculated from sum of the slice areas (7 slices in all) × thickness (2 mm).

Histology

After ischemia the rats were received intracardiac perfusion of 0.9% buffered saline followed by 10% neutral buffered formalin. The brain was then removed and kept in the same fixative for 4 days. Brains were then embedded in paraffin and cut into 8 mm sections. The sections were stained with cresyl-violet. The lesion sites (striatum and cortex) were confirmed microscopically in each brain.

Measurement of active avoidance

The active avoidance was determined for evaluation of memory using active avoidance apparatus (Muromachi Kikai Co., Japan). This apparatus consists of two compartments: light and dark compartment. Each compartment was separated by guillotine doors. The temporal components of the avoidance schedule were an intertrial interval of 29 seconds and a warning period of 9 seconds. Visual (3 seconds) and auditory stimuli (3 seconds) were used as a warning signal. After this conditioned stimulus, a scrambled electric shocks (1.5 mA for 3 seconds) with visual and auditory stimuli through the floor grids were given during the training session. If the animals stepped into other compartment within the warning period, the warning signals ceased, the animals was able to avoid the electric shock, and the trial program was returned to starting point of the 29-seconds intertrial interval. One avoidance session, consisting of 40 times training per day, was conducted everyday for 3 days during the training period. The animals showing more than 50% active

avoidance rate was selected to test the active avoidance as the trained animals. The active avoidance rate was expressed as the percentage of correct avoidance responses out of the trial number (40 times). The detection of acquisition memory was started 1 day prior to the induction of ischemia, meanwhile preservation memory was determined 3 days after the induction of ischemia (2 day after reperfusion).

Morris water maze task test

The maze consisted of a right black plastic pool (d=120 cm; h=30 cm). A circular platform (r=10.6 cm) was placed in the northeast quadrant. The water temperature was 22-23°C. Behavioral parameters (the latency time and distance to reach the platform) were recorded with a videotracking system (Viewpoint Inc., France).

Statistics

Data were expressed as mean ± standard error. The data were analyzed with a one way (or two way) analysis of variance followed by either Dunnett's or Bonferroni's method as a *post hoc* test.

RESULTS

Ischemia/reperfusion-induced brain infarction and cell loss

Varying duration of occlusion and reperfusion created different degrees of ischemia infarction volume. First, we determined infarction volume in the brain operated with different times (30, 60 and 90 min) of ischemia with fixed (72 h) reperfusion time. In the 60 min ischemia brain, the infarction was greatly induced (data not shown). In the different duration of reperfusion with fixed (60 min) ischemia time, the maximum infarct (total infarction volume was 180 ± 94 mm³) was induced in the 60 min-ischemia/24 h-reperfusion (Fig. 1). Severe infarction was detected in the cortex and striatum lesions. The maximum infarction volumes were 112 ± 31 mm³ and 75 ± 14 mm³ in the cortex region and the striatum region, respectively in the 60 min-ischemia/24 h-reperfusion. The number of surviving pyramidal cell in the striatum and the cortex lesions was significantly decreased in the ischemia/reperfusion brain compared to that in the sham-operated brain (Fig. 2).

Active avoidance

Fig. 3A showed the active avoidance rates during the training period and the 15-day examination period after treatment. The mean avoidance rates before the induction of ischemia/reperfusion were 78.4 ± 4.2 (sham-operated group) and 75.6 ± 4.7 (ischemia group), respectively. The active avoidance was not significantly different between the trained groups prior to the induction of ischemia.

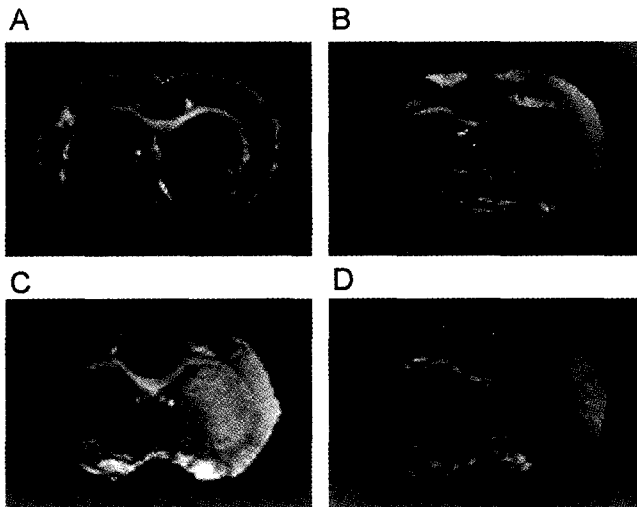


Fig. 1. Brain infarction by different duration of reperfusion by the occlusion of middle cerebral artery in Wistar rats. The brain infarction was detected in brain slices cut at 7 mm away from frontal pole by 2,3,5-triphenyltetrazolium chloride staining methods as described in MATERIALS AND METHODS. A; sham control, B; 60 min-ischemia/12 h-reperfusion, C; 60 min-ischemia/24 h-reperfusion, D; 60 min-ischemia/48 h-reperfusion.

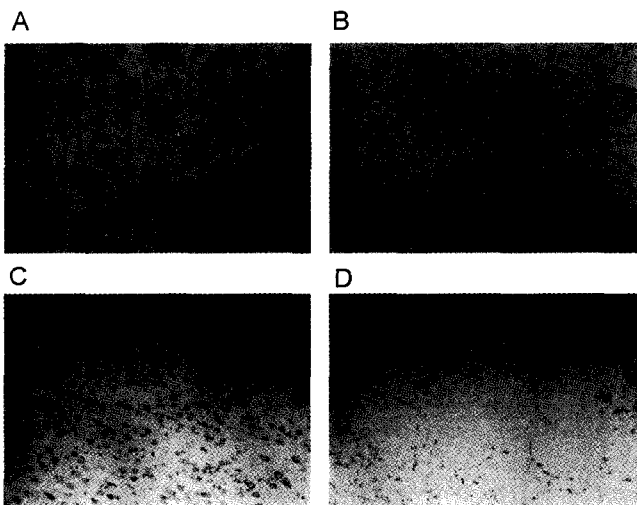


Fig. 2. Pyramidal cell loss in the brain of Wistar rats by the ischemia/reperfusion. Cell loss was detected by the cresyl violet staining method as described in MATERIALS AND METHODS. A; striatum of sham-operated control, B; striatum of 60 min-ischemia/24 h group, C; cortex of sham-operated control, D; cortex of 60 min-ischemia/24 h-reperfusion group

While the sham-operated group kept the consistent level of active avoidance, the active avoidance after the induction of ischemia (from day 4) was significantly decreased compared to the sham-operated group (two way ANOVA test followed by Bonferroni's test as *post hoc*

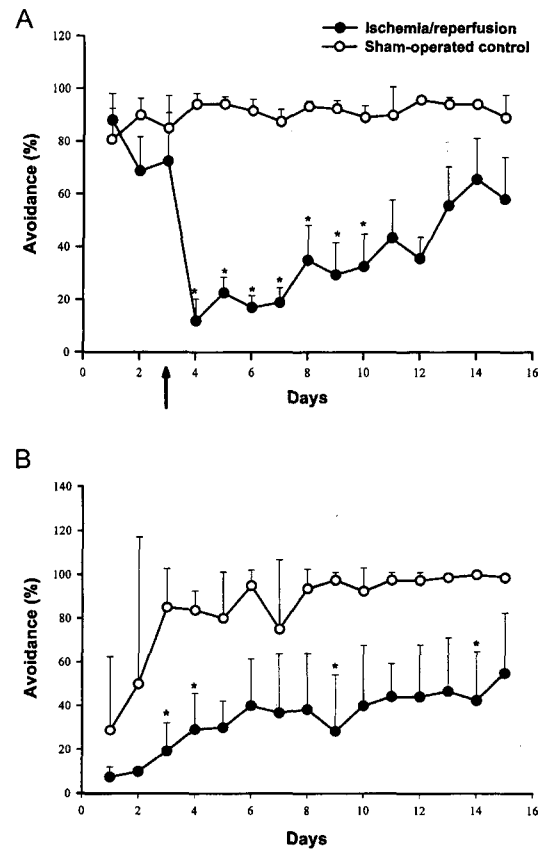


Fig. 3. Changes of the active avoidance response. Active avoidance responses were monitored for evaluation of the preservation (A) and the acquisition memory (B) in the Wistar rats after 60 min-ischemia/24 h-reperfusion brain injury by the middle cerebral artery occlusion (n=8) as described in the MATERIALS AND METHODS. Ischemia was performed on the third training day (A) or 24 h prior to the initial training day (B) as indicated by an arrow. *Significantly different from sham-operated control ($p < 0.05$).

test). This significant difference was being until 10 days. In the acquisition memory test, we monitored the active avoidance in the animals between ischemia/reperfusion group and sham-operated group. The animals in the ischemia/reperfusion group would not reach more than 50% of active avoidance until 15 day test period, whereas animals in the sham-operated group showed more than 80% after 3 days. The difference between two groups, however was not statistically significant (Fig. 3B).

Morris water maze task

The distance and time to reach the platform in sham-operated group were significantly decreased by the time. There were significant difference between ischemia/reperfusion group and sham-operated group by 3 or 4 days in the distance and the latency time. Further difference

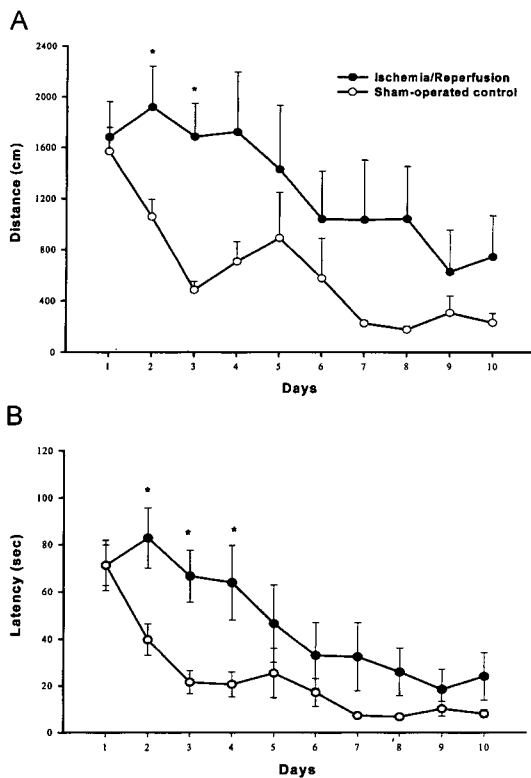


Fig. 4. Changes of the morris water maze task. The distance (A) and latency (B) on the morris maze water task were monitored in the Wistar rats after 60 min-ischemia/24 h-reperfusion brain injury by the middle cerebral artery occlusion ($n=8$) as described in the MATERIALS AND METHODS. *Significantly different from sham-operated control ($p<0.05$).

between groups was not found after day 5 (Fig. 4 A and B).

DISCUSSION

In this study, we found that the acquisition and preservation memory tested by active avoidance were significant difference between the sham and ischemia/reperfusion groups. The water maze acquisition performance was also significant difference between sham and ischemia/reperfusion groups. The infarction volume was increased by the ischemia/reperfusion. Furthermore, severe neuronal damage (pyramidal cell loss) in the striatum and cortex of brain was found in the ischemia/reperfusion brain.

The latency and distance in the active avoidance tested for evaluation of the preservation and acquisition memory were significantly different between ischemia/reperfusion animals and sham-operated animals. Moreover, the difference would not be recovered during the experiment period (until 15 days), while the brain infarction by the ischemia/reperfusion was not detected after 5 day. Therefore, the impairment of the acquisition memory could affect on the preservation memory and two different (the preservation and acquisition memory) aspects of memory seem

not to be separately considered in this study. Similar to the active avoidance, morris water maze task study also showed that the acquisition memory ability was significantly impaired by the induction of ischemia/reperfusion. The impairment of memory corresponds to the induction of brain infarction, especially in the cortex and the striatum lesions. This result is agree with the other findings demonstrating the significant of cortical damage in the impairment of memory function (Takagi *et al.*, 1997; Spangler *et al.*, 1994).

Pyramidal cell loss in the cortex and striatum lesions was also found. Eventhough the number of cells lost in these lesions was not counted and quantitatively analyzed; this study show that behavioral deficits should be related to pyramidal cell damage in the cortex and striatum lesions in the ischemia/reperfusion brain injury. The role of hippocampus and its related structures such as the amygdala and anterior temporal lobe, has been attracted much more attention than other lesions (e.g. cortex lesion) in the formation and preservation of memory (Squire, 1987). In the present study, we showed that in addition to the damage in the hippocampus lesion, damages in the other parts such as the cortex and striatum lesions could contribute to the memory impairment. It was reported that the middle cerebral artery occlusion model used in this study, the cerebral cortex and striatum became infarcted, while the hippocampus was spared (Tamura *et al.*, 1981). It was also reported that middle cerebral artery occlusion in the Sprague-Dawley rat disturbed the retention of memory in the active avoidance response (Hirakawa *et al.*, 1994). No single memory center exists and many parts of the nervous system participate in the representation of a single event, even though memory is distributed throughout various areas of the nervous system. Neocortex could be a memory storage site engaging during the perception, processing and analysis of the materials being learned (Squire, 1987). The possible role of neurochemical system (cholinergic system) in the behavioral changes was demonstrated (Dunnett *et al.*, 1991; Screnin *et al.*, 1991). Moreover, in the middle cerebral artery occlusion, cholinergic fibers from the basal nucleus to the cortex are injured (Kataoka *et al.*, 1991). Together, our results with other findings show that pyramidal cell damage in the cortex lesion may be partially related to memorial disturbance in the ischemia/reperfusion brain injury.

The mechanism by which ischemia/reperfusion-induced brain damages is yet completely answered. Recently, we demonstrated that locomotor activity was increased by ischemia/reperfusion, and the free radical scavenger, green tea extract, inhibited the elevation. We also demonstrated that oxygen radicals generated during the ischemia/reperfusion and/or eicosanoids accumulation are implicated in the brain infarction of the cortex lesion (Hong *et al.*, 1999).

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REFERENCES

- Dunnett, S. B., Everitt, B. J., Robbins, T. W., The basal forebrain-cortical cholinergic system: interpreting the functional consequences of excitotoxic lesions. *Trends Neurosci.*, 14(11), 494-501 (1991).
- Hirakawa, M., Tamura, A., Nagashima, H., Nakayama, H., Sano, K., Disturbance of retention of memory after focal cerebral ischemia in rats. *Stroke*, 25(12), 2471-2475 (1994).
- Hong, J. T., Ryu, S. R., Kim, H. J., Kim, D. B., Lee, J. K., Kim, Y. J., Lee, S. H., Lee, B. M., Kim, P. Y., Protective effect of green tea on the ischemia-reperfusion-induced oxidative brain injury in wistar rats. *Toxicol. Lett.*, 109, 82 (1999).
- Kataoka, K., Hayakawa, T., Kuroda, R., Yuguchi, T., Yamada, K., Cholinergic deafferentation after focal cerebral infarct in rats. *Stroke*, 22(10), 1291-1296 (1991).
- Petito, C. K., Morgello, S., Felix, J. C., Lesser, M. L., The two patterns of reactive astrocytosis in postischemic rat brain. *J. Cereb. Blood Flow Metab.* 10(6), 850-859 (1990).
- Pulsinelli, W., Excitotoxic damage in global ischemia. *Adv. Neurol.*, 71, 62-67 (1996).
- Rolls, E. T., Memory systems in the brain. *Annu. Rev. Psychol.*, 51, 599-630 (2000).
- Scremin, O. U., Jenden, D. J., Effects of middle cerebral artery occlusion on cerebral cortex choline and acetylcholine in rats. *Stroke*, 20(11), 1524-1530 (1989).
- Spangler, E. L., Heller, B., Hengemihle, J., Muthm, N. J., Jones, B. E., Garofalo, P., Ingram, D. K., Thrombosis of parietal, but not striate, cortex impairs acquisition of a 14-unit T-maze in the rat. *Physiol. Behav.*, 56(1), 95-101 (1994).
- Squire, L. R., *Memory and Brain*, Oxford University Press, Oxford, England, pp56-74, 1987.
- Takagi, N., Miyake, K., Taguchi, T., Tamada, H., Takagi, K., Sugita, N., Takeo, S., Failure in learning task and loss of cortical cholinergic fibers in microsphere-embolized rats. *Exp. Brain Res.*, 114(2), 279-287 (1997).
- Tamura, A., Graham, D. I., McCulloch, J., Teasdale, G. M., Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.*, 1(1), 53-60 (1981).
- Yamamoto, M., Tamura, A., Kirino, T., Shimizu, M., Sano, K., Behavioral changes after focal cerebral ischemia by left middle cerebral artery occlusion in rats. *Brain Res.*, 14, 323-328 (1988).