Acta Neurol. Belg., 2010, **110**, 180-185

Cognitive impairment and changes of neuronal plasticity in rats of chronic cerebral hypoperfusion associated with cerebral arteriovenous malformations

Jian HAI*[1], Sao-Hua SU[1], QI LIN*[2], LIN ZHANG[1], Jue-Feng WAN[1], Hui LI[3], Yu-Ying CHEN[3], Yang LU[2]

[1]Department of Neurosurgery, Tongji Hospital, Tongji University, Shanghai 200065, China; [2]Department of Pharmacy, Institutes of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, PR China; [3]Department of Cell Biology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, PR China

Abstract

A new rat model associated with cerebral arteriovenous malformations (AVMs) was developed to study the effect of chronic cerebral hypoperfusion on cognitive function and neuronal plasticity in rats. Aged-matched animals comprised a control group. Three months after surgery, Morris water waze test was performed to evaluate the cognitive function in rats. Neuronal plasticity was assessed by measuring the protein expression of MAP-2, GAP-43 and synaptophysin in the hippocampal regions of rats with immunohistochemistry and western blotting. The average time of escape latency was significantly longer in the model rats than that in the control rats, and both the time spent in the platform quadrant and the frequency of original platform crossing during space probe trials were less than those in the control animals. The expression levels of MAP-2 and synaptophysin protein in hippocampal areas in the model rats were less than those in the control rats. However, there was no difference on the GAP-43 expression between the two groups. These data suggest that chronic cerebral hypoperfusion associated with AVMs could lead to cognitive impairment in rats, which may be partially explained by reduced expression of MAP-2 and synaptophysin at the protein level in the hippocampal area.

Key words: cognition; neuronal plasticity; chronic cerebral hypoperfusion; rat, animal model.

Introduction

Chronic cerebral hypoperfusion represents a common pathophysiological process that usually occurs in conditions such as arteriovenous malformations (AVMs), carotid stenosis/occlusion, dural arteriovenous fistula, artherosclerosis and Alzheimer's disease (1, 2). Sustained cerebral hypoperfusion also accounts for the occurrence of vascular dementia which is characterized by cognitive impairment. Although the effects of acute ischemic insults to the brain are well known (4), the effects of chronic cerebral hypoperfusion (chronic incomplete or non-infarction ischemia) on cerebral function, especially in respect to cognitive function, are poorly delineated.

Clinical evidence has shown that the resolution of chronic cerebral hypoperfusion helps to an improvement of cognitive dysfunction (5), supporting the theory that chronic cerebral hypoperfusion plays a critical role in cognitive impairment. However, the molecular mechanisms of cognitive impairment in chronic cerebral hypoperfusion remain unclear. Learning and memory are important aspects of cognitive function, while the neuronal plasticity plays an essential role in this process (6). The authors investigated the effect of chronic cerebral hypoperfusion on neuronal plasticity in rats by measuring expression of microtubule-associated protein 2 (MAP2), growth-associated protein-43 (GAP-43) and synaptophsin in hippocampal area, approaching its molecule mechanism.

In clinical settings, the relation between chronic cerebral hypoperfusion and cognitive function is difficult to be accessed in humans for several different factors, such as ageing, systematic diseases or educational background, influencing on cognitive performances (3). Experimental studies can help to elucidate this relation because they can use models of pure chronic cerebral hypoperfusion. The rodent models with bilateral common carotid artery occlusion have generally been used to study the states of chronic cerebral hypoperfusion. There are, however, few clinical situations in which the bilateral carotid

*These authors contributed equally to this work.

arteries are totally occluded (7). Based on the hemodynamic characteristics of cerebral AVMs, we established a new rat model of chronic cerebral hypoperfusion by creating a fistula through an end-to-side anastomosis between the right distal external jugular vein (EJV) and the ipsilateral common carotid artery (CCA), followed by ligation of the left vein draining the transverse sinus and bilateral external carotid arteries (ECAs) (8).

Materials and methods

Animal model of chronic cerebral hypoperfusion

All experiments were approved by our institutional Animal Care and Ethics Committee. Model animals with chronic cerebral hypoperfusion were developed as described in detail previously (8). Briefly, 12 Sprague-Dawley rats (200-250 g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg), which was supplemented as needed throughout the procedure. The animals were spontaneously breathing oxygen-enriched room air, and their body temperatures were kept constant with the use of a feedback-controlled heated pad. Under direct magnification, an animal in the model group was produced by creating a fistula via an end-to-side anastomosis with interrupted 11-0 nylon between the right distal EJV and the ipsilateral CCA, followed by ligation of the left vein draining the transverse sinus and the bilateral ECAs. Twelve age-matched rats with ligation of the right EJV and the bilateral ECAs comprised a control group. The rats were housed for 3 months after operation in climate-controlled facilities with 12-hour day/night cycles. They were allowed free access to food and water and were observed daily for abnormalities of activity or diet.

Morris water maze

Three months after operation, the morris water maze task was performed to evaluate the cognitive function in rats of the control and model groups as described previously with minor modification (9). Briefly, the morris water maze consisted of a circular pool, 1.8 m in diameter and 0.6 m in height, filled to a level of 35 cm with water maintained at a temperature of 25 ± 1 °C. The hidden escape platform (diameter: 9 cm) was submerged 2 cm below the surface of the water and was invisible from the water level. Swimming paths were registered by a computerized video imaging analysis system. All rats received four trials per day for four consecutive days with a constant interval of 1 h. The animals were gently placed in water in one of four quadrants, facing the wall of the pool, and the starting quadrant was varied randomly over the trials. Rats were allowed a maximum of 90s to find the escape platform, where it remained for 30s. Rats that failed to locate the platform at the end of 90s were manually guided to the platform. For all training trials, swim speed and escape latency before reaching the platform were measured. All space probe testing consisted of a 60s trial with the platform removed and conducted immediately after the four-day period. Time spent in the target platform location and the number of target crossings over the previous location of the target platform were recorded.

Historial examination

Four rats in the control and model groups were chosen for hematoxylin and eosin (HE) staining after test of the morris water maze. The animals received overdose sodium pentobarbital and transcardinally perfused with 200 ml 0.1 M phosphate buffer saline (PBS, pH7.4) at 4 °C followed by 200 ml 4% buffered paraformaldehyde phosphate. The right brain blocks obtained at coronal level (bregma -3 to -4 mm) from the study animals were removed, post-fixed in the same fixative for 24 h, and then processed for paraffin embedding. Coronal sections (5 mm) were cut and stained with HE.

Immunocytochemistry

Double-labeled fluorescene immunocytochemistry was performed on brain sections (30 μm) at the right coronal level (bregma -3 to -4 mm) from the four rats in control and model groups. The sections were transferred into a 24-well microtiter plate filled halfway with 0.01 M citric acid-sodium citrate buffer, pH 6.0, heated for 10 min in a microwave set to 50% power. The sections were washed with 0.2% Triton-X100-PBS for 5 min. Nonspecific binding sites were blocked with 3% bovine serum albumin in PBS-0.2 Triton-X100 for 30 min and incubated with the polyclonal anti-MAP-2 (Sigma) at a dilution of 1:200 in PBS overnight at 4 °C. The sections were washed three times for 10 min each in PBS at room temperature and incubated in a mixture of fluorescein-labeled anti-rabbit IgG (Dako, Carpinteria, CA, USA) at a dilution of 1:500 and 4 μg /ml propidium iodide (PI, Sigma). The sections were washed three times in PBS, mounted on glass slides, and coverslipped using Gelvatol. The slides were analyzed on a LSM 510 META laser-scanning confocal microscope (Zeiss).

Western blotting

After the remained 4 rats in control and model groups anesthetized deeply, hippocampi were dissected and then homogenized in 2-3 ml of buffer (20 mM Tris-HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 25 μg/ml aprotinin, 25 μg/ml leupeptin, 1 mM $Na_4P_2O_7$, 500 μM phenylmethylsulfonyl fluoride, 4 mM para-nitrophenylphosphate, and 1 mM sodium orthovanadate). Protein concentrations were determined using the BCA protein determination assay (BCA protein assay reagent; Pierce, Rockford, IL). Samples (40 mg protein) were resolved on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked in 5% dry-milk solution for 1 h and then incubated overnight at 4 °C with polyclonal anti-MAP-2, GAP-43 and synaptophysin (1:400, Dako, Carpinteria, CA, USA). Members were washed in Tween-TBS (TTBS) buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20) and incubated with a horseradish peroxidase linked anti-rabbit secondary antibodies according to the primary antibody. The membranes were again washed in TTBS before being developed using the enhanced chemiluminescence method (Amersham Pharmacia Biotech, Arlington Heights, IL). Densitometric analysis of the bands was performed using KS 400 image analysis system (version 3.0; Karl Zeiss, Oberkochen, Germany).

Statistical analysis

The data were expressed in mean ± standard error of the mean (SEM). One-way analysis of variance (ANONA) and paired Student's *t* test were used to evaluate differences in experimental control and model values. An analysis with a value of $p < 0.05$ was considered to be statistically significant.

Results

Cognitive impairment caused by chronic cerebral hypoperfusion

The hidden-platform version of the Morris water maze, a hippocampus-dependent task, requires an animal to learn and remember the relationships between multiple distal cues and the platform location to escape the water (10). As shown in Table 1, rats in the model group have a significant impairment in escape latency during the four-day training as compared to the control rats ($p < 0.05$). The average swim speed during training was not different between the two groups (data not shown), indicating that swimming motivation and ability were similar between the model and control animals, and that the observed differences in learning acquisition were not due to sensorimotor disturbances.

To assess spatial memory more directly, the rats were subject on the day after the end of training to a

Table 1

Average escape latency at different time in the control and model groups, 3 months after operation (mean ± M, n = 12)

Group	Time			
	1st day	2nd day	3rd day	4th day
Control	62.13±14.64	51.26±8.49	48.22±16.60	42.09±15.83
Model	78.82±13.40*	66.69±10.61*	75.82±20.65*	74.01±15.17*

* $P < 0.05$; compared with the control group.

Table 2

Time spent in the target quadrant and the number of target crossings over the target quadrant during the space probe test in the control and model groups (mean ± SEM, n = 12)

Group	test	
	Time spent in the target quadrant	The number of target crossings
Control	24.23±6.48	1.63±1.17
Model	14.89±2.65*	0.54±0.45*

* $P < 0.05$; compared with the control group.

space probe trial in which the platform was removed, and the rats were allowed 60 s to search. As shown in Table 2, there was a significant difference in time spent in the target quadrant between the model and control rats ($p < 0.05$). The rats in the model group spent less swim time in the target platform position. In addition, the model rats also crossed the previous platform position significantly less times as compared to the control rats ($p < 0.05$). These results indicate that the rats of chronic cerebral hypoperfusion have an impairment in learning and memory capacity.

Historial examination

In gross examination of all regions of the rat brains stained with HE under light microscopy in the model group, no evidence of infarction was apparent.

Effect of chronic cerebral hypoperfusion on MAP-2 expression by immunocytochemistry

The brain sections were double-labeled with a polyclonal anti-MAP-2 antibody (green) and PI (red). Neuronal MAP-2 immunoreactivity was characterized by labeling of both soma and dendrites and the nuclei of hippocampal neurons was identified with PI staining. Compared with the control hippocampi, MAP-2 immunoreactivity decreased significantly in rats of the model group (Fig. 1).

Effect of chronic cerebral hypoperfusion on MAP-2, GAP-43 and synaptophysin expression by western blotting

We carried out Western blot analysis to determine whether altered expression levels of MAP-2, GAP-43 and synaptophysin protein were involved. As shown in Fig. 2, chronic cerebral hypoperfusion led to significantly decreased protein levels of MAP-2 and synaptophysin in the hippocampal regions of the model rats compared with the control rats. However, no difference was observed in GAP-43 expression within the hippocampal regions between the control and model rats.

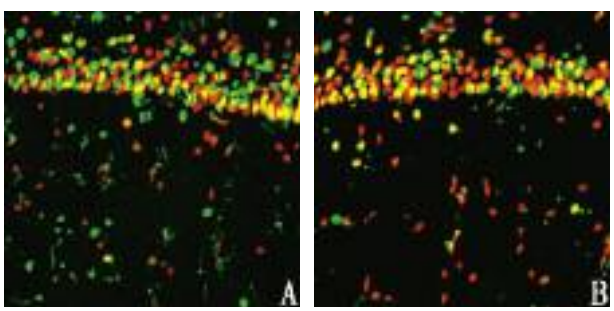

Fig. 1. — MAP-2 immunoreactivity (green) counterstained with PI (red) in the happocampal region of the control (A) and model rats (B) (magnification × 200).

Discussion

The pathophysiological processes occurring in patients with large long-standing cerebral AVMs, artherosclerosis or severe bilateral carotid stenoses are probably linked to chronic noninfarctional hypoperfusion (1, 11). Although inflammation may be another contributing factor being at least partly responsible for the neuropathological changes in rat brain of chronic cerebral hypoperfusion (12), relatively little is known about its impact on cognitive function. In this study, we demonstrated that chronic cerebral hypoperfusion in the rat model associated with AVMs results in cognitive impairment, including learning and spatial memory capacity assessed by the morris water maze.

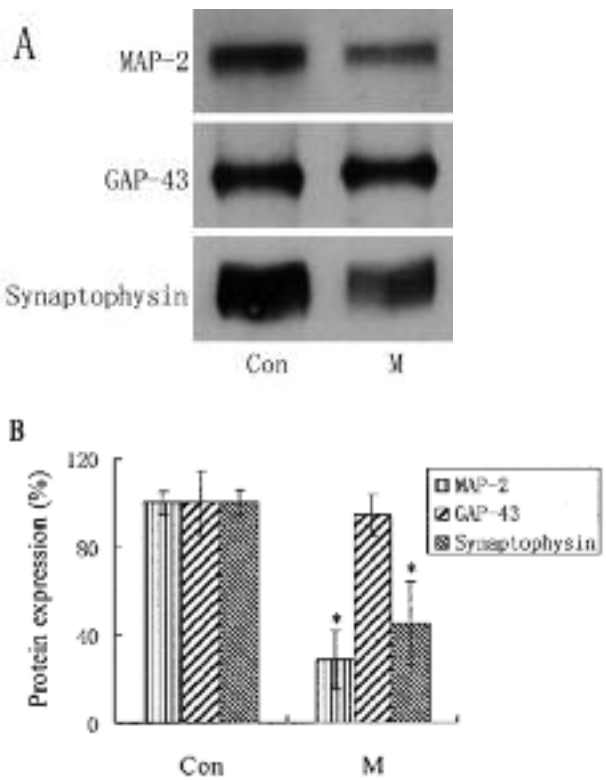

Fig. 2. — Representative western blotting for MAP-2, GAP-43, and synaptophysin expression (A) of the hippocampal regions in the control (Con) and model (M) rats. The protein levels of MAP-2, GAP-43, and synaptophysin expression are quantified by densitometric analysis (B). Data represent mean ± SEM (n = 4). *$p < 0.05$ vs. the control group.

Primary hemodynamic alterations in patients with cerebral AVMs decrease cerebral perfusion pressure (CPP) in the adjacent brain as a result of intracranial arteriovenous shunting (13). Several animal models have been developed in an attempt to recreate the hemodynamic changes that occur in cerebral AVMs. In the model described by Spetzler et al., venous drainage is primarily orthograde into the systemic circulation, so that intracranial venous hypertension is underemphasized (14). In the model described Morgan et al., anastomosis of the distal carotid artery to the distal external jugular vein produces an arteriovenous fistula fed via retrograde flow through the internal carotid artery, which also very likely causes venous hypertension. Their model, however, does not decrease systemic mean arterial pressure when the fistula is opened (15). Based on the fundamental hemodynamic characteristics of human AVMs, we modified that animal model by creating a fistula via an end-to-side anastomosis between the distal EJV and the ipsilateral CCA to induce sustained systemic arterial hypotension. In addition, this method caused occlusion of the contralateral vein draining the transverse sinus, which led to intracranial venous hypertension, and ligation of bilateral ECAs caused the occlusion of blood from extracranial arteries (8). As a result, both systemic arterial shunting and venous outflow restriction contributed to significant reductions in the whole CPP including the hippocampal area in rats. Our previous and present studies showed that no areas of infarction were observed by light microscopic examination in this model (16, 17).

MAP-2 is a cytoskeletal protein mainly localized to dendrites of neurons. The expression of MAP-2 coincides with dendritic outgrowth, branching and post-lesion dendritic remodeling, suggesting that this protein is involved in the neuronal plasticity (18-. A diminished immunoreactivity of MAP-2 has been found to be a sensitive marker of ischemic damage after permanent and transient focal stroke (19). In the present study, both immunohistochemistry and western blotting also demonstrated the MAP-2 down-regulation in the hippocampal regions in rat model of chronic cerebral hypoperfusion associated with AVMs. It is very likely that a decrease in MAP-2 expression causes abnormal interactions of cytoskeletal proteins and/or abnormal assembly of microtubules and in turn may affect neuronal dysfunction with impaired learning behavior and spatial memory.

Synaptophysin is a 38 kDa calcium-binding glycoprotein which lies on the membrane of presynaptic vesicles, and takes part in the development and plasticity of nerve synapses (20). The level of synaptophysin protein usually reflects the synaptic numbers and density. Our data here showed that chronic cerebral hypoperfusion could induce down-regulation of the protein level of synaptophysin, indicating that its expression may be one of the molecular mechanisms behind learning and memory deficits after chronic cerebral hypoperfusion associated with AVMs. Moreover, synaptophysin is a constituent of neurotransmitter-containing presynaptic vesicle membranes (21) and a decrease in synaptophysin may influence neurotransmission which could be responsible for cognitive impairment.

GAP-43 is an intracellular membrane-associated protein expressed in neuronal growth cones, and invloves in axonal growth, synaptogenesis, synaptic remodeling and neurotransmitter release (22). The expression of GAP-43 protein is closely related to nervous system development, synapse plasticity and neuronal regeneration, and plays an important role in guiding axon growth and regulating formation of new contact on axon (23). When a synapse forms, the protein level of GAP-43 expression is down-regulated. However, there is no altered GAP-43 expression in the hippocampal regions in the model rats compared to the control rats, which may be a compensatory mechanism for chronic cerebral hypoperfusion (20).

In summary, we have investigated the effect of chronic cerebral hypoperfusion associated with AVMs on cognitive function and found that this kind of chronic noninfarction ischemia led to cognitive impairment. Our experimental data suggest some interesting molecular mechanisms underly such a pathophysiological condition. Down-regulation of MAP-2 and synaptophysin protein expression in the hippocampus of rats may partially account for the impairment of learning and memory induced by chronic cerebral hypoperfusion associated with AVMs. No altered GAP-43 expression in the hippocampus in the model rats may reflect the compensatory and remodeling mechanism for chronic cerebral hypoperfusion.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Grant NO: 30772233 to J. Hai).

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Jian Hai MD PhD,
Department of Neurosurgery,
Tongji Hospital, Tongji University,
389 Xin-Cun Road,
Shanghai 200065, China
E-mail: haijiandoc@yahoo.com.cn