

Efficacy of a combination of taurine and stenosis removing on cognitive impairment induced by carotid artery stenosis in rats

X.-Z. LIU¹, F.-C. WANG², Y. WANG³, J.-X. ZHANG⁴

¹Department of Community Medicine, The Second Hospital of Shandong University, Shandong, China

²Department of General Surgery, Shandong, China

³Department of Medical Engineering, Thoracic Hospital of Shandong Province, Shandong, China

⁴Department of Traditional Chinese Medicine, The Second Hospital of Shandong University, Shandong, China

Abstract. – OBJECTIVE: We assessed the protective effect on a carotid artery stenosis rat model with using a combination of taurine and stenosis treatment. Rats were randomly divided into five groups: Group 1 (sham), Group 2 (model), Group 3 (stenosis removing), Group 4 (taurine), and Group 5 (combination).

MATERIALS AND METHODS: The carotid artery stenosis model was created by ligating the common carotid artery along with various gauges of needle and then removing the needle. The cognitive performance was analyzed using P300 latency and escape latency in the Morris water maze (MWM) task. The levels of oxidative products malondialdehyde (MDA), inflammatory factors interleukin (IL-1 β) and tumor necrosis factor (TNF- α), the activities of anti-oxidative enzymes superoxide dismutase (SOD) and catalase (CAT) were determined using enzyme-linked immunosorbent assay (ELISA) kits, respectively.

RESULTS: The results revealed that the cognitive impairment can be reduced by stenosis removing and taurine, combining the administration of stenosis removing and taurine had a significant effect on the recovery of cognitive function.

CONCLUSIONS: A further study showed that, the protection of cognitive function is related to inhibition of oxidative stress and suppression of inflammatory response.

Key Words:

Carotid artery stenosis, Taurine, Stenosis removing, Cognitive impairment, Oxidative stress, Inflammatory response.

Introduction

Carotid artery stenosis is a major public health issue and negatively affects the quality of life of patients and their families¹. Clinical studies² have shown that severe carotid artery stenosis often

leads to symptoms related to the transient ischemic attack and ischemic stroke. Moreover, carotid artery stenosis may be an independent risk factor for cognitive impairment and decline³⁻⁵. Previous studies^{6,7} have documented that the damaged neurological function would be improved when carotid artery stenosis was relieved. Some key factors have been implicated in the pathogenesis of carotid artery stenosis. Among them, oxidative stress and inflammatory response have been identified. Brain oxidative stress was considered to be the major risk factor in the pathogenesis of vascular dementia⁸. Furthermore, Shalhoub et al⁹ has shown that inflammation is crucial in promoting the development and progression of carotid artery stenosis. Therefore, the potent antioxidant and anti-inflammatory agents' interference may be beneficial in the treatment of carotid artery stenosis induced cognitive impairment. Taurine (2-aminoethylsulfonic acid), which is a sulfur-containing β -amino acid, is ubiquitously distributed in animal tissues and cells, accounts for approximately 0.1% of total human body weight¹⁰. Taurine (T) has a wide variety of physiologic effects and plays an important role in various essential biological processes^{11,12}. T is an antioxidant and has tissue protective effects in oxidant-induced injuries¹³. Taurine reacts with hypochlorous acid (HOCL) to produce taurine chloramines (TauCL), which is a powerful regulator of inflammation and it regulates the pro-inflammatory mediators in inflammatory cells¹⁴. Thus, we postulated that Taurine would protect carotid artery stenosis induced cognitive impairment. In the present work, we assessed the effectiveness of a combination of taurine and stenosis removing in a rat model to determine its potential as a novel clinical treatment for cognitive impairment induced by carotid artery stenosis.

Materials and Methods

Animals

Adult male Wistar rats (weighing 280-300 g) supplied by Sichuan University Experimental Animal Center. The animals were housed in a room under temperature (22-25°C), with a relative humidity of 50±10%, 12:12 h day/night cycle and free access to water and food. If no special instructions, the reagents used in experimental methods are purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Experimental Procedure

The rat model of severe carotid artery stenosis was established by a method described previously¹⁵. Briefly, the rats were fasted overnight and freely accessed to water. The sutures were bathed in dexamethasone solution for 10 min before the surgery. The rat was fixed after 10% chloral hydrate anesthesia, and neck midline incision was performed. The skin and muscles were bluntly dissected, and the bilateral common carotid artery was dissociated. A syringe needle with diameter of 0.45 mm and common carotid artery was tied into a slipknot at 0.5 cm from the bifurcation of internal and external carotid artery of the proximal part of common carotid artery using #0 surgical suture. Rats were given aspirin as an anticoagulant (30 mg/L) in their drinking water 3 days after surgery. Two weeks after the model was successfully established, the experimental animals were randomly allocated into five groups: Group 1 (sham), Group 2 (model), Group 3 (stenosis removing), Group 4 (taurine), and Group 5 (combination). Each group consisted of 12 rats. In the sham group, the bilateral common carotid artery was exposed, but no ligation was made. In the stenosis removing and combination groups, the rats were given an operation to relieve carotid stenosis at 14 day after the occurrence of carotid stenosis. 100 mg/kg taurine (Tianjin Chase Sun Pharmaceutical Co., Ltd., Tianjin, China) suspended in saline was gavage administrated to the rats in taurine and combination groups once per day for consecutive 4 weeks. The same volume of physiological saline was injected into the rats in sham, model, and stenosis removing groups.

Stenosis Rate

Four rats from each group were anesthetized by excessive chloral hydrate and their common carotid arteries were isolated and fixed with 4%

paraformaldehyde. The stenosis of each common carotid artery was cut out to prepare the cross-sectional slices. Three different sections were selected from the stenosis and the area stenosis rate of each section was analyzed. The mean value of area stenosis rate for each rat was calculated.

Detection of the P300 Auditory Event-related Potential

Rats were fixed on the stereotaxic apparatus, eliminated the fur of the head, behind the ears, and the butt of tail. Then 3.3 cm detection electrodes (Medicid 3E, Beijing Newco Company, Beijing, China) were pierced to the scalp of the intersection of sagittal suture and two-plane connection. The P300 wave was recorded and documented in charts.

Morris Water Maze Test

The rats were subjected to a water maze test (Shanghai Jiliang Software Technology Co. Ltd., Shanghai, China), which consisted of a circular water tank (2 m diameter and 1.5 m high) filled with water (25±1°C). Animals were tested for place-learning acquisition with the escape platform (5 cm diameter) located in the middle of the southeast quadrant, 3.5 cm below water surface. Training was performed four times daily for 5 consecutive days. The rats were trained to swim randomly from each of the four starting positions while facing the wall, and allowing them to swim freely until they found the platform. The rats were given 2 min to find the platform and to remain on it for 15 s. The rats were guided to the platform and left there for 15 s if failing to find the platform within 2 min. To evaluate the rat's spatial retention ability, on the 6 day, the platform was removed and allowing each rat to swim freely for 2 min inside the pool. The total swimming distance traveled and the swimming distance in the target quadrant for 2 min were recorded by the tracking system (ANY-maze video tracking system, Stoelting Co., Wood Dale, IL, USA).

Measurement of Biomarkers of Oxidative Stress

After Morris water maze test, the rats were euthanized by an overdose of intraperitoneal pentobarbital, the hippocampus were isolated quickly. Then prepared as a 5% (w/v) tissue homogenate in 0.9% saline solution. The ho-

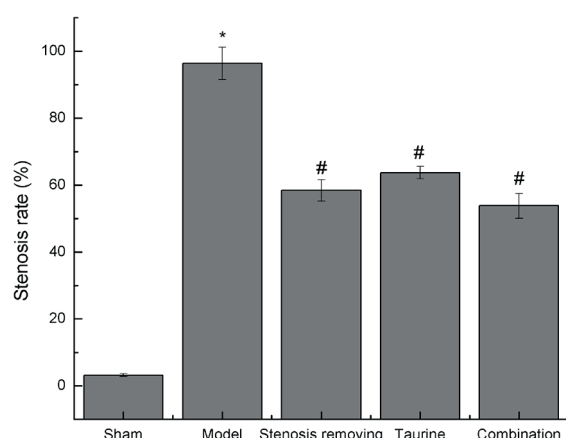


Figure 1. The changes of the rate of carotid stenosis in rats in each group. Data were shown as mean $\pm$ SD. * $p < 0.05$ vs sham group, # $p < 0.05$ vs model group.

mogenate was centrifuged and the supernatant was collected. The levels of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Nanjing Jiancheng Co., Nanjing, China).

Measurement of Inflammatory Factor Level

The concentrations of interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) were measured by ELISA kits (Nanjing Jiancheng Co., Nanjing, China).

Histopathological Examination

The rats were anesthetized by excessive chloral hydrate and the skulls were opened to expose the hippocampus. The hippocampus was isolated and fixed with 4% paraformaldehyde for 48 h, followed by dehydration, paraffinization, embedding, and hematoxylin and eosine (H&E). The sections were observed under light microscope (DP73; Olympus, Tokyo, Japan).

Statistical Analysis

Statistical analysis was performed using SPSS statistical package version 18.0 (IBM, Armonk, NY, USA). All data were reported as the mean $\pm$ SD. Student's *t*-test was used as appropriate for comparison between different groups. $p < 0.05$ was considered statistically significant.

Results

Stenosis Rate

Figure 1 illustrates how the stenosis rate changes in each group. The stenosis rate of the model group was significantly higher than that of the sham group ($p < 0.05$). The stenosis rate of the model group was also significantly higher than that of the taurine group ($p < 0.05$). The combined use of stenosis removing and taurine produced the best result in terms of stenosis rate.

P300 Auditory Event-Related Potential

The P300 latencies of the sham group were shorter than the model group ($p < 0.05$). Compared with the model group, the P300 latencies of the stenosis removing and taurine groups were significantly shorter (all $p < 0.05$). Furthermore, combined therapy achieved a better result than single treatment with stenosis removing or taurine (Figure 2).

Morris Water Maze Test Analysis

The escape latency to find the hidden platform and swimming distance in the target quadrant in all rats were shown in Figure 3. The escape latency in both the stenosis removing and taurine groups tended to decrease over-time. Furthermore, the results revealed that the combination group presented a shortest latency to find the platform.

Compared with the model group, the swimming distance percentage in the target quadrant was significantly increased in the steno-

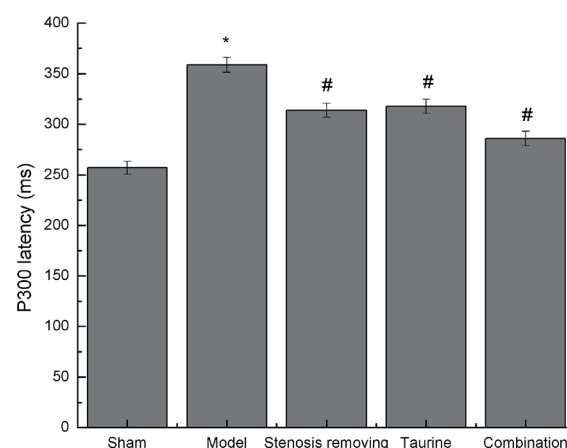


Figure 2. The changes of P300 latency in each group. Data were shown as mean $\pm$ SD. * $p < 0.05$ vs. sham group, # $p < 0.05$ vs. model group.

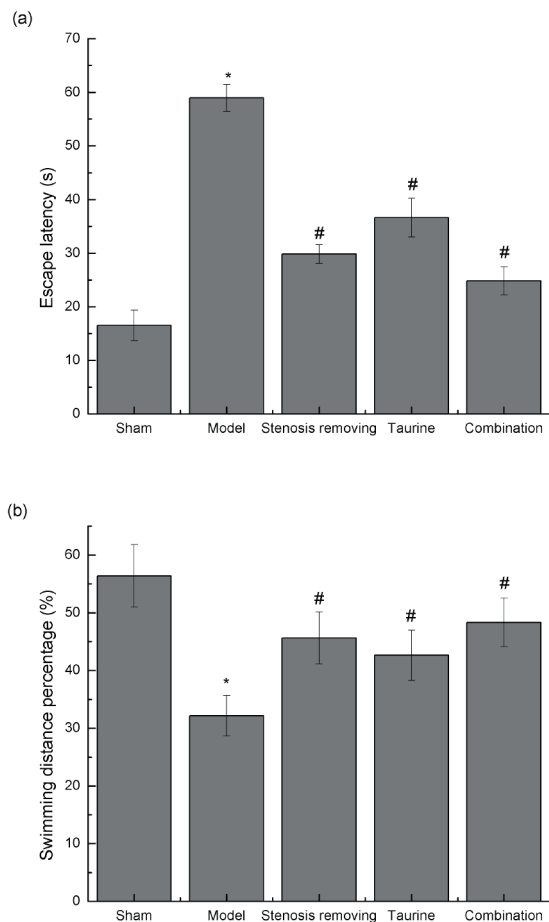


Figure 3. he changes of escape latency to find the hidden platform (a) and swimming distance percentage in the target quadrant (b) in water maze test. Data were shown as mean $\pm$ SD. * $p < 0.05$ vs sham group, # $p < 0.05$ vs model group.

sis removing and taurine groups (all $p < 0.05$). Furthermore, the greatest significant increase in the swimming distance percentage in the target quadrant was detected in the combination group.

Hippocampus MDA, SOD, and CAT Analysis

As shown in Figure 4a, the MDA level was significantly higher in the model group compared with the other groups (all $p < 0.05$). Stenosis removing or taurine-treatment kept MDA at a lower level than in the model group. However, stenosis removing + T controlled MDA better than stenosis removing or taurine alone. The SOD and CAT levels were lowest in the model group and highest in the combination group. Treatment with stenosis removing or taurine

attenuated the decline in the level of SOD and CAT. However, combined therapy achieved a better result than single treatment with stenosis removing or T (Figure 4b-4c).

Hippocampus IL-1 β and TNF- α analysis

Statistical differences were observed between the control and the treatment group with regards to hippocampus IL-1 β and TNF- α levels (Figure 5). Stenosis removing led to a statistical decrease in the IL-1 β and TNF- α levels in hippocampus compared with the model group ($p < 0.05$, respectively). Similarly, the T group showed a statistical decline in the IL-1 β and TNF- α levels in hippocampus compared with the model group ($p < 0.05$, respectively). However, the combination group showed the most decrease in IL-1 β and TNF- α levels.

Histopathological Evaluation

Figure 6 shows representative images of H&E stained cord specimens from all groups. In sham group, rat hippocampal neurons in alignment had normal morphology, quantity and distribution with big and round nuclei, as well as clear nucleolus (Figure 6a), while rat hippocampal neurons in control group arranged in disorder with condensed nuclei, disappeared nucleolus and unclear structure in part of neurons (Figure 6b). Rat hippocampal neurons markedly ameliorated in treatment groups compared with those at corresponding time points in control group (Figure 6c-6e). Between the treatment groups, the greatest significant decrease was achieved in the combination group (Figure 6e).

Discussion

Vascular cognitive impairment is a cognitive impairment syndrome from mild cognitive impairment to dementia induced by cerebrovascular disease risk factors and obvious or not obvious cerebrovascular disease¹⁶. A past work¹⁷ has shown that carotid artery stenosis can caused cognitive impairment as an independent risk factor. The detection of the P300 auditory event-related potential and the Morris water maze test demonstrated that the cognitive decline induced by carotid artery stenosis was markedly improved, while used the stenosis removing and the administration of T. Furthermore, we have shown that the combined use of stenosis removing and T produced the

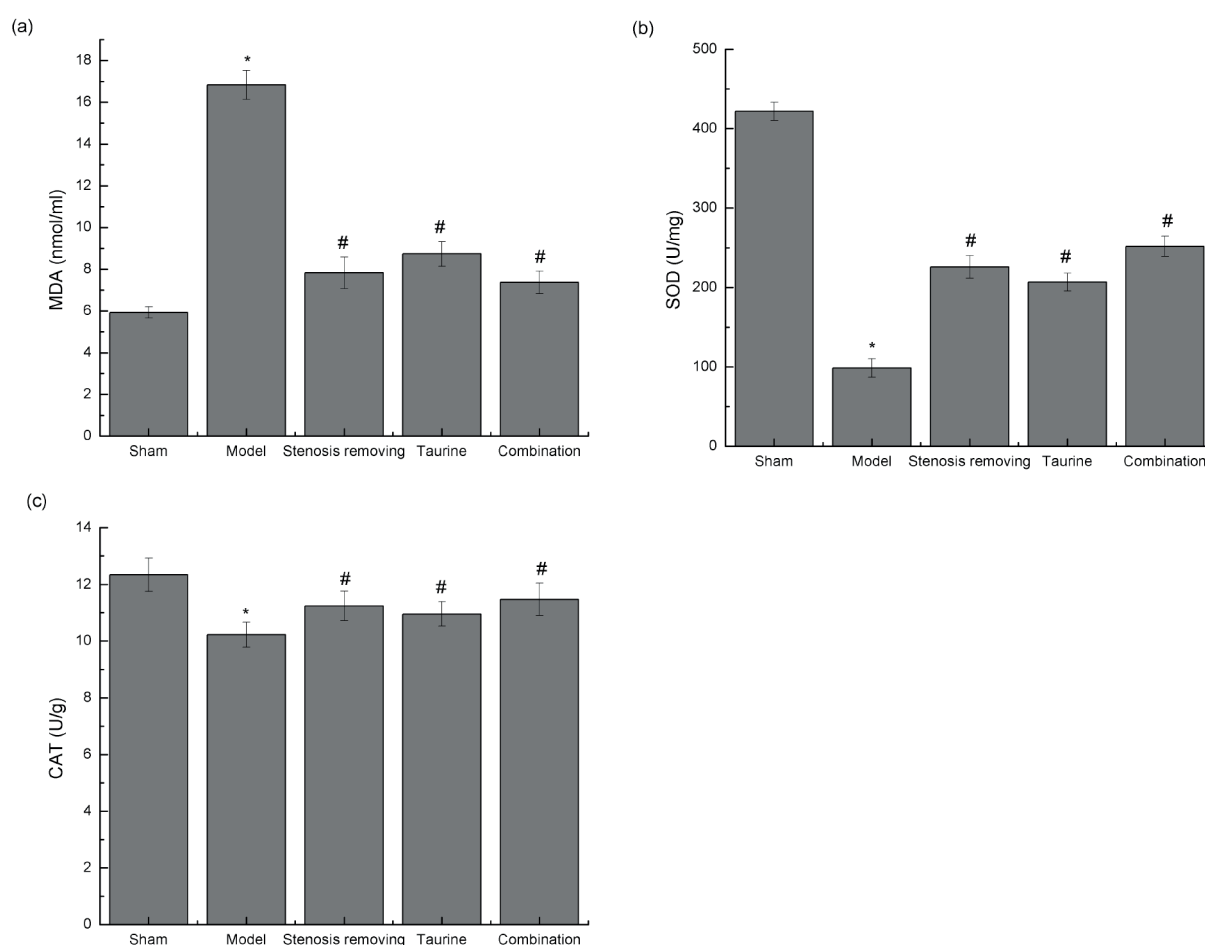


Figure 4. The changes of the levels of MDA (a), SOD (b) and CAT (c) in hippocampus. Data were shown as mean $\pm$ SD. * $p < 0.05$ vs. sham group, # $p < 0.05$ vs. model group.

best result. Animal experimental and clinical study revealed that oxidative stress played an important role in the pathogenesis of brain injury¹⁸⁻²⁰. Some enzymes, such as SOD and CAT, provide cellular protection against damage from oxygen-derived free radicals. SOD is an enzyme used as a biochemical indicator of the pathological states associated with oxidative stress²¹. CAT is an enzyme that plays an important role in reactive oxygen species detoxification, converts hydrogen peroxide into harmless by products²². MDA is the end product of the oxygen-derived free radicals and lipid oxidation, which reflects the damage caused by reactive oxygen species²³. In the experiment, with MDA significantly increased, SOD and CAT markedly decreased in carotid artery stenosis-induced rats. Stenosis removing and the administration of taurine caused a remarkable

reduction in the MDA level and a significant increase in SOD activity and CAT content in hippocampus. This indicates that carotid artery stenosis stimulated oxidative stress, and stenosis removing and the administration of taurine could inhibit oxidative stress via improving the activities of SOD and CAT, decreasing the MDA. Meanwhile, the effect of combined therapy was better than stenosis removing or single taurine-treatments. Inflammatory response is another crucial factor influencing neuronal destiny²⁴. Pro-inflammatory cytokines, IL-1 β and TNF- α are the potential cytokines, which produced by cells of the innate immune system (monocytes, neutrophils, NKT cells), toxic reagents, trauma, oxidative stress, antibodies, or immune complexes²⁵. They can initiate inflammatory reactions and induce expression of other cytokines after cerebral ischemia²⁶. Thus,

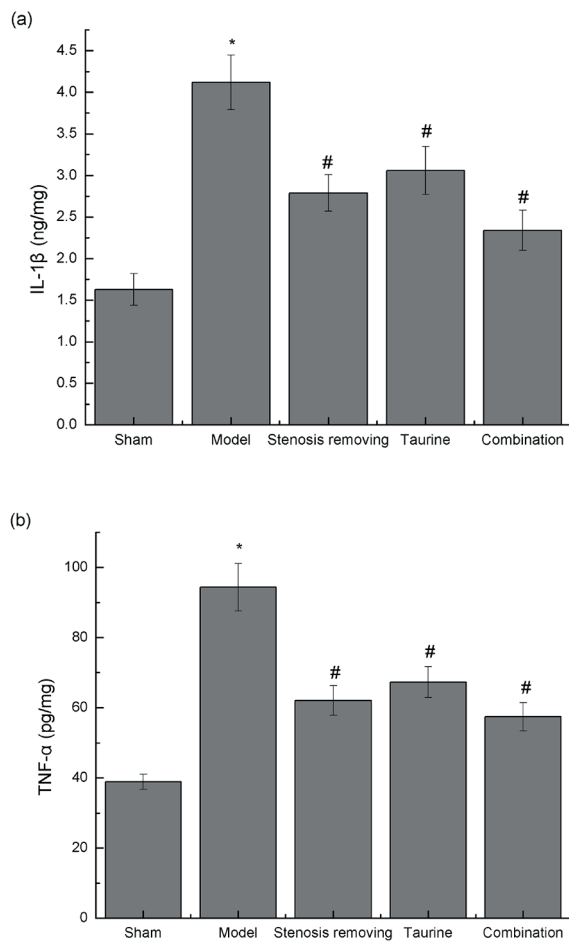


Figure 5. The changes of the levels of IL-1 β (a) and TNF- α (b) in hippocampus. Data were shown as mean $\pm$ SD. * p <0.05 vs. sham group, # p <0.05 vs. model group.

in this report, we determined the levels of IL-1 β and TNF- α in rats. We found that the IL-1 β and TNF- α levels were significantly increased in carotid artery stenosis rats. In contrast, the levels of IL-1 β and TNF- α were significantly decreased in stenosis removing and taurine-treated rats, and the combined use of stenosis removing and taurine had the best result.

Conclusions

The principal findings of the current study are that cognitive impairment can be reversed by stenosis removing and the administration of taurine, the combined use of stenosis removing and taurine had the best result. A further study showed that the protection of cognitive function is related to inhibition of oxidative stress and suppression of inflammatory response.

Ethics Statements

All animal procedures were approved by the Ethical Committee for Animal Experiments, Sichuan University, China.

Conflict of interest

The authors declare no conflicts of interest.

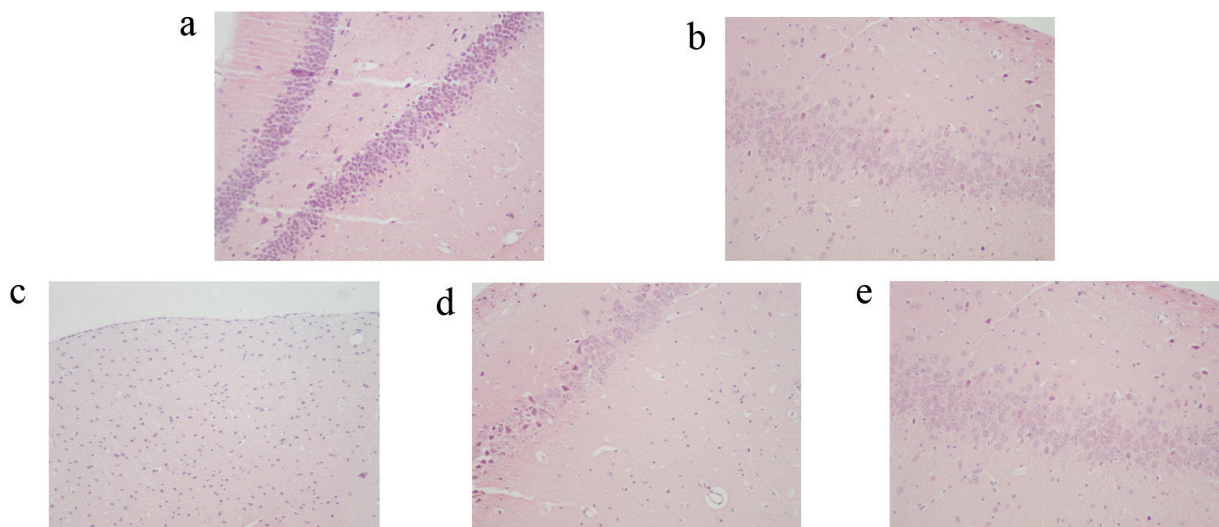


Figure 6. Histopathological photomicrographs of hippocampal tissue. (a) sham group; (b) model group; (c) stenosis removing group; (d) taurine group; (e) combination group ($\times 200$).

References

- 1) TAUSKY P, HANEL RA, MEYER FB. Clinical considerations in the management of asymptomatic carotid artery stenosis. *Neurosurg Focus* 2011; 31: E7.
- 2) YOUNG KC, JAIN A, JAIN M, REPLOGLE RE, BENESCH CG, JAHROMI BS. Evidence-based treatment of carotid artery stenosis. *Neurosurg Focus* 2011; 30: E2.
- 3) JOHNSTON SC, O'MEARA ES, MANOLIO TA, LEFKOWITZ D, O'LEARY DH, GOLDSTEIN S, CARLSON MC, FRIED P, LONOSTRETH WT Jr. Cognitive impairment and decline are associated with carotid artery disease in patients without clinically evident cerebrovascular disease. *Ann Intern Med* 2004; 140: 237-247.
- 4) MATHIESEN EB, WATERLOO K, JOAKIMSEN O, BAKKE SJ, JACOBSEN EA, BØNAA KH. Reduced neuropsychological test performance in asymptomatic carotid stenosis: the Tromsø study. *Neurology* 2004; 62: 695-701.
- 5) SZTRIHA LK, NEMETH D, SEFCSIK T, VECSEI L. Carotid stenosis and the cognitive function. *J Neurol Sci* 2009; 283: 36-40.
- 6) CHEN YH, LIN MS, LEE JK, CHAO CL, TANG SC, CHAO CC, CHIU MJ, WU YW, CHEN YF, SHIN TF, KAO HL. Carotid stenting improves cognitive function in asymptomatic cerebral ischemia. *Int J Cardiol* 2012; 157: 104-107.
- 7) DUAN W, CHUN-QING Z, ZHENG J, GUI L, HUANG HQ, CHEN KN. Relief of carotid stenosis improves impaired cognition in a rat model of chronic cerebral hypoperfusion. *Acta Neurobiol Exp (Wars)* 2011; 71: 233-243.
- 8) RINALDI B, FINICELLI M, DONNICUO M, BERNARDO GD, GRITTI G, GAUDIO SD, FORTE A, PELUSO G, CIPOLLARO M, ROSSI F, GALDERISI U. G-CSF contributes at the healing of tunica media of arteriotomy-injured rat carotids by promoting differentiation of vascular smooth muscle cells. *J Cell Physiol* 2016; 231: 215-223.
- 9) SHALHOUB J, VIIRI LE, CROSS AJ, GREGAN SM, ALLIN DM, ASTOLA N, FRANKLIN IJ, DAVIES AH, MONACO C. Multi-analyte profiling in human carotid atherosclerosis uncovers pro-inflammatory macrophage programming in plaques. *Thromb Haemost* 2016; 115: 1064-1072.
- 10) YU YR, NI XQ, HUANG J, ZHU YH, QI YF. Taurine drinking ameliorates hepatic granuloma and fibrosis in mice infected with *Schistosoma japonicum*. *Int J Parasitol Drugs Drug Resist* 2016; 6: 35-43.
- 11) HUANG Y, LI Z, YANG Z. Roles of ischemia and hypoxia and the molecular pathogenesis of post-burn cardiac shock. *Burns* 2003; 29: 828-833.
- 12) KARABACAK K, KAYA E, ULUSOY KG, SEYREK M, KURTOGLU M, DOGANCL S, YILDIRIM V, YILDIZ O, DEMIRKILIC U. Effects of taurine on contractions of human internal mammary artery: a potassium channel opening action. *Eur Rev Med Pharmacol Sci* 2015; 19: 1498-1504.
- 13) MOTOYAMA T, OKAMOTO K, KUKITA I, HAMAGUCHI M, KINOSHITA Y, OGAWA H. Possible role of increased oxidant stress in multiple organ failure after systemic inflammatory response syndrome. *Crit Care Med* 2003; 31: 1048-1052.
- 14) SCHULLER-LEVIS GB, PARK E. Taurine and its chloramine: modulators of immunity. *Neurochem Res* 2004; 29: 117-126.
- 15) YUAN Y, GUO Q, YE Z, PINGPING X, WANG N, SONG Z. Ischemic post conditioning protects brain from ischemia/reperfusion injury by attenuating endoplasmic reticulum stress-induced apoptosis through PI3K-Akt pathway. *Brain Res* 2011; 1367: 85-93.
- 16) HACHINSKI VC, BOWLER JV. Vascular dementia. *Neurology* 1993; 43: 2159-2160.
- 17) SZTRIHA LK, NEMETH D, SEFCSIK T, VECSEI L. Carotid stenosis and the cognitive function. *J Neurol Sci* 2009; 283: 36-40.
- 18) LI ZY, LIU B, YU J, YANG FW, LUO YN, GE PF. Ischemic post conditioning rescues brain injury caused by focal ischemia reperfusion via attenuating proteins oxidation. *J Int Med Res* 2012; 40: 954-966.
- 19) TAFFI R, NANETTI L, MAZZANTI L, BARTOLINI M, VIGNINI A, RAFFAELLI F, PASQUALETTI P, VERNIERI F, PROVINCIALI L, SILVESTRINI M. Plasma levels of nitric oxide and stroke outcome. *J Neurol* 2008; 255: 94-98.
- 20) NANETTI L, RAFFAELLI F, VIGNINI A, PEROZZI C, SILVESTRINI M, BARTOLINI M, PROVINCIALI L, MAZZANTI L. Oxidative stress in ischemic stroke. *Eur J Clin Invest* 2011; 41: 1318-1322.
- 21) ERTEN SF, KOC AK, OZDEMIR I, AYDEMIR S, COLAK A, REEDER BS. Protective effect of melatonin on experimental spinal cord ischemia. *Spinal Cord* 2003; 41: 533-538.
- 22) ILHAN A, YILMAZ HR, ARMUTCU F, GUREL A, AKYOL O. The protective effect of nebivolol on ischemia/reperfusion injury in rabbit spinal cord. *Prog Neuropsychopharmacol Biol Psychiatry* 2004; 28: 1153-1160.
- 23) QIAN H, LIU D. The time course of malondialdehyde production following impact injury to rat spinal cord as measured by microdialysis and high-pressure liquid chromatography. *Neurochem Res* 1997; 22: 1231-1236.
- 24) BARONE FC, FEUERSTEIN GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999; 19: 819-834.
- 25) OZSARLAK-SOZER G, SEVIN G, OZGUR HH, YETIK-ANACAK G, KERRY Z. Diverse effects of taurine on vascular response and inflammation in GSH depletion model in rabbits. *Eur Rev Med Pharmacol Sci* 2016; 20: 1360-1372.
- 26) KADHIM HJ, DUCHATEAU J, SEBIRE G. Cytokines and brain injury: invited review. *J Intensive Care Med* 2008; 23: 236-249.