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Therapeutic Effect of Tetramethylphrazine on Cognitive Impairment After Subarachnoid Hemorrhage: An Experimental Study

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Tetramethylphrazim is an alkaloid monomer purified from Chinese herb Chuanxiong (rhizome of Ligusticum wallichii). Tetramethylphrazim can improve micro-circulation and increase cerebral blood flow in the ischemic tissue and has been widely used in the treatment of cardio and cerebral ischemic diseases. We tried to evaluate the therapeutic effect of tetramethylphrazim on cognitive impairment after subarachnoid hemorrhage in a rat model. 70 SD rats were randomly divided into control group, subarachnoid hemorrhage group and tetramethylphrazim-treated group. The subarachnoid hemorrhage model was induced by an endovascular filament. The capability of learning and memory was tested by Morris water maze. The level of superoxide dismutase (SOD) in brain tissue was assayed by a xanthine oxidase assay kit with a spectrophotometer. The subarachnoid hemorrhage group exhibited seriously spatial learning and memory impairment in both place navigation trail test and spatial probe trial. In place navigation trail test the mean values of escape latency of tetramethylphrazim-treated group were shorter than those of subarachnoid hemorrhage group ($p < 0.05$). In spatial probe trial, there were significant differences in the frequency of crossing the former platform site between tetramethylphrazim-treated group and subarachnoid hemorrhage group ($p < 0.05$). In tetramethylphrazim-treated group, compared with subarachnoid hemorrhage group, the activity of SOD increased significantly ($p < 0.05$). In conclusion, tetramethylphrazim could improve spatial learning and memory impairment caused by subarachnoid hemorrhage and the raising of activity of SOD in brain tissue may be involved in its mechanism.

Key Words: Tetramethylphrazine, Subarachnoid hemorrhage, Morris water maze, Learning, Memory.

INTRODUCTION

Nowadays, the morbidity and mortality of subarachnoid hemorrhage (SAH) have decreased greatly and most survivors have even recovered in physical and neural function[1]. However, it is also proved that cognitive impairment including memory, attention and thinking persistently exist following subarachnoid hemorrhage[2].

Tetramethylphrazim (TMP) is an alkaloid monomer purified from Chinese herb Chuanxiong (rhizome of *Ligusticum wallichii*). Tetramethylphrazim has been proved to have a lot of biological functions. It can improve micro-circulation and increase cerebral blood flow in the ischemic tissue and has been widely used in the treatment of cardio and cerebral ischemic diseases[3]. But its effect on subarachnoid hemorrhage is still unknown. Some reports indicated tetramethylphrazim could prevent and treat the cerebral vasospasm secondary to subarachnoid hemorrhage[4]. Cerebral vasospasm could lead to wide cerebral ischemia, which is an important reason for cognitive impairment[5]. In present study, we tried to understand the effect of tetramethylphrazim on cognitive impairment after subarachnoid hemorrhage with a rat model.

EXPERIMENTAL

70 Adult male SD rats, weighing 200-300 g, were divided into 3 groups randomly, which were control group (10 rats), subarachnoid hemorrhage group (30 rats) and tetramethylphrazim-treated group (30 rats). All experiments received approval by the Animal Care Committee of the Medical Faculty of Zhejiang University, China.

Animal preparation: All rats were fasted for 12 h before surgery. The animals were intubated and mechanically ventilated with 0.8 % halothane in 70 % N_2O and 30 % O_2. A thermostatically regulated, feedback-controlled heating pad was used to maintain rectal temperature at 37 ºC. A two-channel laser-Doppler Flowmeter (LDF, MBF3D, Moor Instruments Ltd., UK) was used for continuous monitoring of the local cortical blood flow (LCBF) of each hemisphere in the area of the cerebral cortex, supplied by the middle cerebral artery (MCA). To allow placement of the LDF probes, bilateral burr holes

with a diameter of 1 mm were drilled 5 mm lateral and 1 mm posterior to the bregma without any injuries to the dura mater. Then the animals were placed in supine position with the head firmly fixed in a stereotactic frame. A local cortical blood flow probe was positioned in each burr hole using a micromanipulator. The local cortical blood flow was continuously measured from 0. 5 h before the induction of subarachnoid hemorrhage until 1.5 h after it.

Induction of subarachnoid hemorrhage: Subarachnoid hemorrhage was induced by use of the endovascular puncture method as described[6]. Briefly, after identifying the right carotid artery, a 3-0 nylon filament was advanced *via* the external carotid artery into the internal carotid artery (ICA) until decrease of the ipsilateral LCBF, as indicated by the LDF. This ensures a positioning of the tip of the filament near the bifurcation of the ICA, occluding the origin of the MCA. Then the filament was pushed 3 mm further in order to perforate the ICA near its intracranial bifurcation. Then the suture was withdrawn into the external carotid artery, reperfusing the ICA and producing subarachnoid hemorrhage. The endovascular occlusion lasted a maximum of 5 min in all cases. In the control group the filament was only put into the external carotid artery and then withdrawn 5 min later.

Drug: In the tetramethylphrazim-treated group, tetramethylphrazim (Beijing Fourth Medicine Factory, 960427) (25 mg/kg/day) was injected intraperitoneally just after the induction of subarachnoid hemorrhage for 14 days. In the control and subarachnoid hemorrhage group only saline was injected.

Water maze: To evaluate the effects of tetramethylphrazim on learning and memory deficits induced by subarachnoid hemorrhage, Morris water maze test was employed. The water maze consisted of a tank of water made opaque by the addition of powdered milk (0.5-1.5 %, 22-25 ºC), 1.2 m in diameter and 0.5 m in height, divided clockwise into four quadrants A, B, C and D. One centimeter below the surface of the water, a rigid platform, 11 cm in diameter, was fixed in Quadrant A. The surviving rats were trained for 4 days on negotiating spatial bias,receiving 6 trials everyday. Time taken (escape 1atency) to find the platform was recorded for each trial for 2 min, while the mean latency of 6 trials everyday was considered as its mean escape latency. The platform was removed on day 5 and the spatial probe trial was conducted. Rats were allowed to swim freely for 2 min in the absence of the platform and the frequency of crossing the former platform was recorded.

Measurement of superoxide dismutase (SOD) activity in brain tissue: The mice were sacrificed by decapitation after the completion of the water maze test. The cerebral cortex and hippocampus were rapidly separated and homogenized in 9 volume of ice-cold phosphate buffered saline (PBS) (0.05 mol/L, pH 7.4). The homogenate was centrifuged at 3600 rpm for 10 min and the supernatant was collected to determine the activity of SOD, according to the methods provided by the xanthine oxidase assay kit directions. The absorbance was measured at 550 nm by a spectrophotometer (Unico 7202B, USA).

Statistical analysis: A statistical analysis was performed using one-way ANOVA, Mortality was analyzed with the Chi-square test. Differences were considered significant at the $p < 0.05$ level. Results are presented as mean ± SD.

RESULTS AND DISCUSSION

Mortality: The mortality was not significantly reduced from 60 % in subarachnoid hemorrhage group to 56.7 % in tetramethylphrazim-treated group.

Water maze test: With the increase of training days, the escape latency time to find the platform in the maze decreased step by step in different experimental groups. The mean escape latency in the control group was 29.2 ± 3.5 s at the 1st day, 11.4 ± 2.9 s at the 2nd day, 9.1 ± 2.7 s at the 3rd day and 8.7 ± 1.8 s at the 4th day. The latency was 50.2 ± 7.6, 20.6 ± 5.3, 22.6 ± 4.6 and 18.3 ± 4.9 s in subarachnoid hemorrhage group rats, while 46.1 ± 7.8, 15.5 ± 4.4, 14.8 ± 4.1 and 13.1 ± 3.6 s in tetramethylphrazim group. The mean escape latency in the control group rats (n = 10) was significantly shorter than those in tetramethylphrazim (n = 13) and subarachnoid hemorrhage group (n = 12) everyday ($p < 0.05$), while the latency in the tetramethylphrazim group rats was significantly shorter than those in subarachnoid hemorrhage group at the 2nd, 3rd and 4th day ($p < 0.05$) (Fig. 1). The prolonged time for finding the platform in subarachnoid hemorrhage group and tetramethylphrazim group suggested that subarachnoid hemorrhage could induce learning and memory impairment. Administration of tetramethylphrazim could attenuate the learning and memory dysfunction caused by subarachnoid hemorrhage.

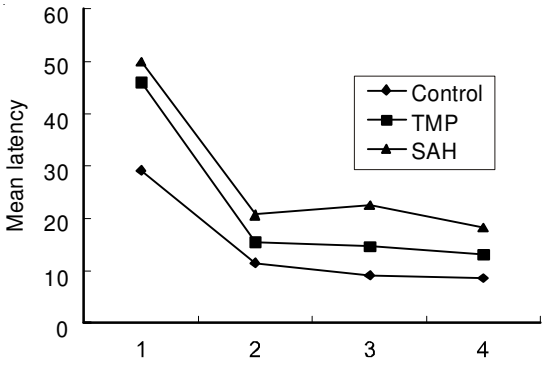

Fig. 1. Mean escape latency in the control group rats (n = 10) was significantly shorter than those in tetramethylphrazim (n = 13) and subarachnoid hemorrhage group (n = 12) everyday ($p < 0.05$), while the latency in the tetramethylphrazim group rats was significantly shorter than those in subarachnoid hemorrhage group at the 2nd, 3rd and 4th day ($p < 0.05$)

During the spatial probe trial, the control rats and tetramethylphrazim rats spent the majority of their time swimming in the quadrant where the platform had been located, while the subarachnoid hemorrhage rats distributed their time more uniformly in all four pool quadrants. The frequency of crossing the former platform within 2 min was 12.2 ± 1.5 in control group (n = 10), 5.3 ± 1.4 in subarachnoid hemorrhage group (n = 12) and 8.7 ± 2.5 in tetramethylphrazim group (n = 13). There was statistical difference between subarachnoid hemorrhage group and tetramethylphrazim group ($p < 0.05$) (Fig. 2).

Level of superoxide dismutase (SOD) in brain tissue: The SOD activity was 64.3 ± 7.5 in the tetramethylphrazim group (n = 13), 50.2 ± 8.6 in the subarachnoid hemorrhage group (n = 12) and 77.3 ± 5.7 in the control group (n = 10).

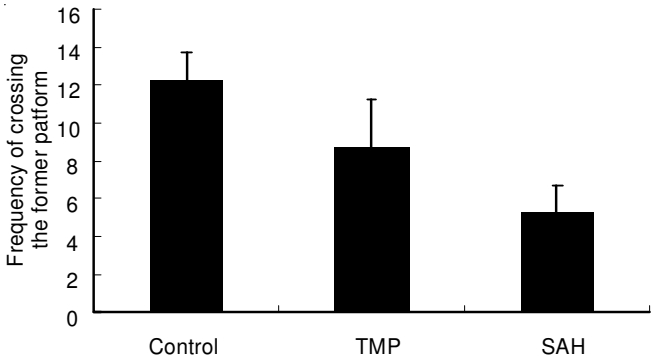

Fig. 2. During the spatial probe trial, the frequency of crossing the former platform within 2 min wasin tetramethylphrazim group. There was statistical difference between subarachnoid hemorrhage group and tetramethylphrazim group ($p < 0.05$)

Increased SOD activity was observed in tetramethylphrazim group, as compared with subarachnoid hemorrhage group ($p < 0.05$) (Fig. 3).

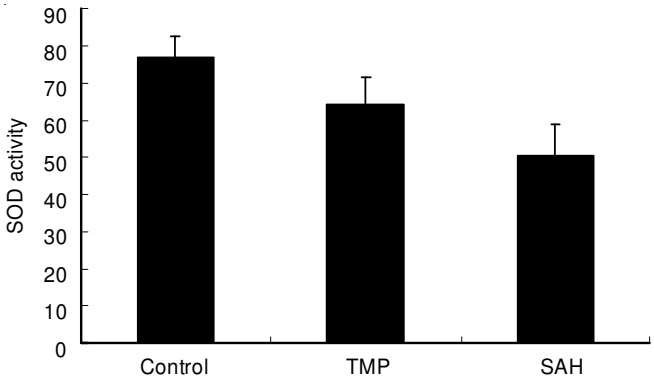

Fig. 3. Superoxide dismutase activity was 64.3 ± 7.5 in the tetramethylphrazim group (n = 13), 50.2 ± 8.6 in the subarachnoid hemorrhage group (n = 12) and 77.3 ± 5.7 in the control group (n = 10). Increased SOD activity was observed in tetramethylphrazim group, as compared with subarachnoid hemorrhage group ($p < 0.05$)

Subarachnoid hemorrhage is a devastating illness with a 30-day mortality rate of 30-40 %. Although most survivors are free of physical handicap, approximately 50 % remain permanently disabled because of cognitive dysfunction[7]. So, more attentions should be paid to improve the cognitive deficits in the therapy scheme for subarachnoid hemorrhage patients.

Some experiments had proved that tetramethylphrazim could improve microcirculation and attenuate oxidative stress[3,8,9]. It was reported that tetramethylphrazim can improve impairment of learning and memory after cerebral ischemic injury[10]. And we tried to explore the protective effect of tetramethylphrazim on cognitive impairment following subarachnoid hemorrhage.

In this experiment, the escape latency in tetramethylphrazim-treated group is shorter and the time spent in the target quadrant is longer than that in subarachnoid hemorrhage group, which proved that early application of tetramethylphrazim could prevent learning and memory impairment after subarachnoid hemorrhage.

Superoxide dismutase is an important antioxidase that can scavenge superoxide radicals. Lowered SOD activity was observed in subarachnoid hemorrhage group and tetramethylphrazim group, as compared with the control group, which indicated subarachnoid hemorrhage could result in the superoxide radicals accumulation. In the tetramethylphrazim group rats, higher level of SOD was also observed compared with subarachnoid hemorrhage group, which in thylphrazim might alleviate the oxidative injury by increasing the SOD activity.

After subarachnoid hemorrhage, erythrocytes in the subarachnoid space lyse with releasing of oxyhemoglobin (OxyHb). Oxyhemoglobin autoxidizes to methemoglobin and releases superoxide anion radicals[10]. Superoxide anion radicals lead to neuronal damage by initiating and propagating lipid peroxidation, protein breakdown and DNA damage that in turn leads to cellular apoptosis and endothelial injury to vessels[11-13]. Some authors found that oxidative stress could result in widespread endothelial dysfunction[14]. The long-term cognitive dysfunction following subarachnoid hemorrhage is probably the result of diffuse microvascular endothelial dysfunction and secondary cerebrovascular insufficiency[14]. So treatment strategies aimed at improving microcirculation and scavenging oxidative stress hold the best promise for improving cognitive outcomes after subarachnoid hemorrhage.

Conclusion

Tetramethylphrazim could prevent learning and memory impairment secondary to subarachnoid hemorrhage. The mechanism may be associated with increasing SOD activity and attenuating oxidative stress.

REFERENCES

1. R.A. Bornstein, B.K.A. Weir, K.C. Petruk and L.B. Disney, *Neurosurgery*, **21**, 651 (1987).
2. B.O. Hutter and J.M. Gilsbach, *Neurosurgery*, **33**, 999 (1993).
3. Y.H. Shih, S.L. Wu, W.F. Chiou, H.H. Ku, T.L. Ko and Y.S. Fu, *Neuroreport*, **13**, 515 (2002).
4. G. Shao, S. Bao and B. Chu, *Nervous Dis. Ment. Hyg.*, **3**, 265 (2003).
5. C. Bermueller, S. C. Thal, N. Plesnila, R. Schmid-Elsaesser, U. Kreimeier and S. Zausinger, *J. Neurolog. Sci.*, **241**, 73 (2006).
6. J.B. Bederson, I.M. Germano and L. Guarino, *Stroke*, **26**, 1086 (1995).
7. W.T. Longstreth Jr., L.M. Nelson and T.D. Koepsell and G. van Belle, *Neurology*, **43**, 712 (1993).
8. G.E. Yao, L.Z. Wang and M.E. Chen, *Chin. J. Clin. Rehabil.*, **7**, 1877 (2003).
9. C.F. Liu, M.H. Lin, C.C. Lin, H.W. Chang and S.C. Lin, *J. Biomed. Sci.*, **9**, 299 (2002).
10. R.L. Macdonald, K. Bryce and A. Weir, *Stroke*, **22**, 971 (1991).
11. R.E. Ayer and J.H. Zhang, *Acta Neurochir.*, **S104**, 31 (2008).
12. W.R. Markesbery, *Free Radic. Biol. Med.*, **23**, 134 (1997).
13. K. Takata, H. Sheng, C.O. Borel, D.T. Laskowitz, D.S. Warner and F.W. Lombard, *Exp. Neurol.*, **213**, 336 (2008).
14. Z. Zhang, T. Wei, J. Hou, G. Li, S. Yu and W. Xin, *Eur. J. Pharmacol.*, **467**, 41 (2003).