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A RODENT MODEL OF CEREBRAL VASCULAR DEMENTIA AND DRUG ACTION

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There have reports suggested that cerebral blood flow (CBF) has decreased in patients with both senile dementia of the Alzheimer's type and multi-infarct dementia, which are characterized by marked cognitive impairments. In addition, recent studies have demonstrated that decrease of CBF precedes the onset of multi-infarct dementia. These findings further suggest that chronic reduction of CBF may play an important role in the formation and progression of cerebral vascular dementia. Although transient cerebral ischemia, based upon vascular "reperfusion", is apparently not paralleling the clinical condition, the transient cerebral ischemia model is one of the major methods investigated and the other is the cerebral embolism operation. Cognitive impairment and neuronal damages have been fully studied using these transient and/or embolic ischemia models. There are, however, few investigations focused the attention on the influence of chronic decrease of CBF on cognitive processes. In the present study, we have chosen a chronic ischemic model which is produced by permanent occlusion of bilateral common carotid arteries (2VO) in rats to investigate the neuronal damage and cognitive deficits through radial maze performance. We investigated furtherly the effects of tetramethylpyrazine (TMP), a constituent isolated from *Ligusticum Chuanxiong* on such a model.

1. Mortality after the permanent 2VO

Under pentobarbital anesthesia, bilateral common carotid arteries of the Wistar rat were carefully separated from the cervical sympathetic and vagal nerves through a ventral cervical incision. Then the arteries were doubly ligated with silk sutures simultaneously in the permanent 2VO group. The animals received the same surgical operation without carotid artery ligation was used as sham-operated control. Permanent 2VO caused high mortality in 7 weeks old rats and about 71% of the animals died within 24 hr after the ligation. We found that the mortality of permanent 2VO rats decreased with age and was less than 10% in rats 13 weeks old and over. The CBF in the cerebral cortex decreased nearly to 80% of the control 3 weeks after the permanent 2VO. Therefore, the rats older than 13 weeks were used for the subsequent experiments.

2. Histological changes

One and 4 months after the permanent 2VO, rats were anesthetized with pentobarbital and perfused intracardially with a heparinized

phosphate buffer followed by 4% paraformaldehyde. The excised brains were postfixed and used for histological examination. The infarction in the cerebral cortex, striatum, hippocampus and hypothalamus and the rarefaction in white matter were measured. Various degrees of lesion were detected in ischemic rat brain. Although the number and size of the lesioned area differed among individual animals, histological changes in the cerebral cortex, striatum and in white matter were found in over half of the animals tested. The ratio of animals with infarction in the cerebral cortex 1 and 4 months after permanent 2VO operation were 28.6% and 14.3%, respectively, whereas those in the striatum 1 and 4 months after the permanent 2VO both were 42.9%. Infarction was not observed in the hippocampus or hypothalamus. On the other hand, the rarefaction in white matter was found in 14.3 and 71.4% of the animals examined 1 and 4 months after the permanent 2VO, respectively. A significance of difference was found between the control and 2VO group 4 months after the operation. The number of CA1 neurons significantly decreased 4 months but not 1 month after the operation. In contrast, the number of neuronal cells in the CA2 and CA3 subfields did not significantly decrease even 4 months after the permanent 2VO.

3. Acetylcholine levels in the brain tissues following permanent 2VO

Rats were killed by microwave irradiation 1 and 4 months after permanent 2VO. The cerebral cortex, striatum, hippocampus and hypothalamus were rapidly dissected out and was homogenized in 1 ml of 0.2N perchloric acid solution containing 2 nmol ethylhomocholine as an internal standard. The acetylcholine and choline levels of the water layer were determined using HPLC equipped with an electrochemical detector. ACh content in the striatum significantly decreased 1 month after the permanent 2VO by 14.9%, while those in other regions were not changed by the operation. On the other hand, the ACh contents in the cortex and hypothalamus as well as in the striatum significantly decreased 4 months after the 2VO. The choline contents in these regions also significantly decreased.

4. Eight-arm radial maze learning and memory

We used an 8-arm radial maze task which was one of the most commonly used methods for testing spatial learning and memory in rodents. The rats failed to learn the radial maze performance 3 - 21 days after the permanent 2VO. The number of errors of 2VO rats did not decrease with repeated training. The rats, that had been well pretrained, exhibited only slight impairment in the retention of radial maze performance 3 - 7 days after the permanent 2VO operation. The number of errors in the pretrained permanent 2VO group gradually decreased to the level of the sham-operated control group with trials.

Following recovery to the level of the control at the 8 - 10th post operative days, a 0- or 3-min delay was interposed on alternating days. The interposition of the delay significantly increased the number of errors in the pretrained 2VO group, but not in the control. Four months after the permanent 2VO, the radial maze performance was carried out again without the delay. The pretrained rats with 2VO showed impairment in this stage compared with the sham-operated control.

5. Effect of TMP on the 2VO-induced disruption of the 8-arm radial maze learning performance

The rats, that had not been pretrained, failed to learn the radial maze performance 3-21 days after the permanent 2VO (the rats received 15 learning trials). Administration of TMP (3 and 10 mg/kg, i.p.) patially but significantly attenuated the 2VO-induced impairment of maze learning task. In the retention task, permanent 2VO slightly impaired the maze performance of the pretrained rats. Treatment of the 2VO rats with TMP 10 mg/kg did not attenuate this impairment.

Present results demonstrate that TMP has beneficial effects on spatial cognitive impairment in rats with progressive brain infarction. TMP is known to increase the cerebral blood flow and to protect against severe acute ischemic attack, resulting in increase of the survival rate after the ischemia in Mongolian gerbils. Moreover, TMP produces the vasodilatory action by inhibiting both Ca^{2+} influx and intracellular Ca^{2+} release. These pharmacological effects of TMP may contribute to the improvement of the cognitive impairment induced by chronic decrease of CBF. The present results demonstrated the cognitive deficits and neuropathological changes following chronic cerebral ischemia in rats. The permanent 2VO is not only a useful model for studying the behavioral and neuropathological changes of human vascular dementia, but also for developing effective drugs for the treatment of this disease. TMP may be served as one of the candidates for dementia treatment.

6. References

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