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Huperzine A improves cognitive deficits caused by chronic cerebral hypoperfusion in rats

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Abstract

The effects of (−)-huperzine A, a promising therapeutic agent for Alzheimer's disease, on learning behavior and on alterations of the cholinergic system, the oxygen free radicals and energy metabolites induced by permanent bilateral ligation of the common carotid arteries were investigated in rats. Daily oral administration of huperzine A produced a significant improvement of the deficit in the learning of the water maze task, beginning 28 days after ischemia, correlating to about 33–40% inhibition of acetyl-cholinesterase activity in cortex and hippocampus. Huperzine A significantly restored the decrease in choline acetyltransferase activity in hippocampus and significantly reduced the increases in superoxide dismutase, lipid peroxide, lactate and glucose to their normal levels. The present findings demonstrate that the improvement by huperzine A of the cognitive dysfunction in the late phase in chronically hypoperfused rats is due to its effects, not only on the cholinergic system, but also on the oxygen free radical system and energy metabolism. Our results strongly suggest that huperzine A has therapeutic potential for the treatment of dementia caused by cholinergic dysfunction and/or decrease of cerebral blood flow.

Keywords: (−)-Huperzine A; Acetylcholinesterase; Cholinesterase inhibitor; Cerebral ischemia; Morris water maze; Learning; Memory; Free radical; Alzheimer's disease

1. Introduction

Dementia has become a major public health issue as the proportion of elderly increases in the population. Apart from Alzheimer's disease, the most common dementia in the elderly, vascular dementia is also a common clinical syndrome of intellectual decline produced by ischemia, hypoxia, or haemorrhagic brain lesions. Previous studies focusing on the pathogenetic mechanism have revealed that, like the pathosis of Alzheimer's disease, cholinergic abnormalities are associated with the disturbance of cognitive function in patients with vascular dementia (Gottfries et al., 1994). Tohgi et al. reported that in patients with vascular dementia of the Binswanger type or multiple small infarct type, acetylcholine concentration in the cerebrospinal fluid was significantly lower than in the controls, and showed a significantly positive correlation with dementia scale scores (Tohgi et al., 1996). These findings introduced the possibility of using cholinergic substances as therapeutic intervention in patients with vascular dementia. It is conceivable that inhibition of brain acetylcholinesterase to increase the synaptic concentration of acetylcholine may improve the cognitive dysfunction and neuropathology in patients suffering from cerebral ischemia dementia.

Recent studies have demonstrated that the decreases in cerebral blood flow precede the onset of vascular dementia (Roman et al., 1993). Reduced cerebral blood flow also lessens the availability of glucose and other metabolic substrates, and this chronically impaired neuronal energy production may initiate and sustain the cascade of neuropathological events that underlie this dementia (Beal et al., 1993). Permanent bilateral ligation of the common carotid arteries in rats is a chronic cerebral hypoperfusion model, which results in significant reduction of cerebral blood flow (Tsuchiya et al., 1993) and causes learning and memory impairments and neuronal damage resembling those in Alzheimer's disease and cerebrovascular disease

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(Ni et al., 1995). The hypoperfused rat provided us with a useful model to understand the pathophysiology of chronic cerebrovascular disorders.

There are several acetylcholinesterase inhibitors used in Alzheimer's disease therapy. Among these agents, huperzine A, a novel alkaloid isolated from the Chinese herb *Huperzia serrata (Thunb) Trev*, has been proven to be one of the most promising agents for palliative treatment of Alzheimer's disease, based on its centrally active and long-lasting inhibition of acetylcholinesterase, high bioavailability and minimal side-effects (Tang and Han, 1999). Previous studies showed that, in addition to its potent acetylcholinesterase inhibitory effect, huperzine A also exhibited neuroprotective effects on glutamate-induced cytotoxicity (Ved et al., 1997) and corrected the pathological changes in oxygen free radicals in Alzheimer's disease patients (Xiao et al., 1999). Taken together, these findings have prompted us to explore whether huperzine A has beneficial effects in the cerebral ischemia model. Improving effects of long-term treatment with huperzine A on learning deficits and brain neuronal damage induced in rats by permanent bilateral ligation of the common carotid arteries are reported.

2. Materials and methods

2.1. Chemicals

(−)-Huperzine A (colorless crystals, purity > 98%) isolated from *Huperzia serrata* was prepared by the Department of Phytochemistry in the Shanghai Institute of Materia Medica.

2.2. Animals

The male rats used in the present study were from the Sprague–Dawley strain, weighing 195–205 g at the beginning of the experiments. They were housed in groups of three or four in a room maintained at 23 ± 1°C with a 12-h light–dark cycle and were allowed free access to water and food.

2.3. Surgery

The rats were anesthetized with pentobarbital–Na (40 mg/kg, i.p.). The bilateral common carotid arteries were exposed and gently separated from the carotid sheath and vagus nerve. In rats assigned to the ischemic groups each artery was ligated with a 5–0 silk suture. As sham-operated controls, another group of rats received the same operation without ligation.

2.4. Water maze task

The water maze was adapted from the Morris water task. The test apparatus comprised a circular pool placed in a room with symbols such as triangles, circles, waves, and rectangles on the pool wall. The pool was 150 cm in diameter, 60 cm deep, and was filled to a height of 35 cm with water at room temperature 23 ± 1°C to cover a black platform (diameter 10 cm). The water was darkened with 50 ml of prepared Chinese ink. Four points at the edge of the pool, each equidistant from its neighbors, that divided the pool into four quadrants (North, South, East and West) were designated as start positions. The platform was submerged approximately 15 mm below the surface of the water and remained in the middle of the West quadrant (Q4) for the entire training period. Swimming activity of each rat was monitored by a video camera linked to a computer through an image analyzer. Behavior was tested on 5 consecutive days, with each rat receiving four trials a day. Within each block of four trials, a rat started at each of the starting locations randomly. The time taken to find the platform (escape latency) was measured and was averaged over four trials. If a rat failed to find the platform within 60 s, it was placed on the platform. Regardless of whether the rat found the platform or not, it was kept there for 20 s. There was a 30-s recovery period between trials. The probe trial (one trial without platform) was assessed immediately after the fifth block of four trials, and the time spent in the quadrant (Q4) where the platform had been set during training was recorded.

2.5. Experimental design

For the behavioral study, the hypoperfused rats were divided randomly into two groups, each with 8–11 animals of much the same mean body weights 2 weeks after surgery. Daily oral administration of huperzine A 0.1 mg/kg, twice per day or its vehicle (saline) was started on day 15 and terminated on the day of killing (day 33). During the behavioral test, the drug was administered 40 min before the trials. The water maze task was begun on day 29 after surgery.

2.6. Biochemical examinations

To evaluate cholinergic activity, oxygen free radical system and metabolites in the rat brain, the rats were decapitated 40 min after the last administration. The brain was separated on ice into three regions (cortex, hippocampus, and striatum). Each region was weighed and homogenized in 9 vol ice-cold saline and the homogenate was diluted with an appropriate buffer solution for determination of the relative biochemical index. Enzyme activity or their product levels were described as below. Choline acetyltransferase activity was determined by measuring the rate of formation of acetylcholine from acetyl-CoA, using the radiochemical method (Fonnum, 1975). Acetylcholinesterase activity was assayed using a spectrometric method (Ellman et al., 1961). The assay for superoxide dismutase activity was based on its ability to inhibit the

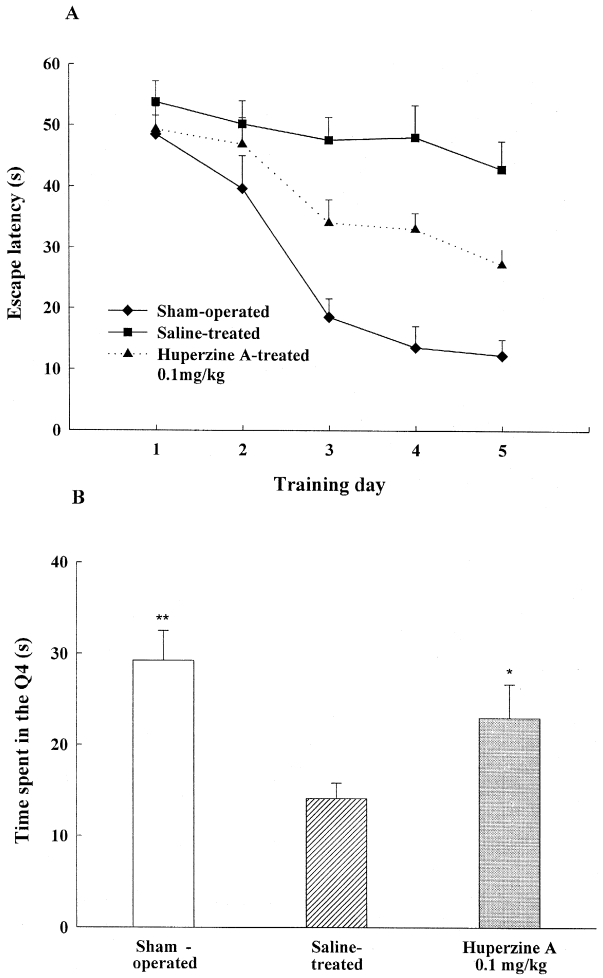

Fig. 1. Effects of huperzine A on water maze performance deficits caused by permanent bilateral ligation of the common carotid arteries in rats. The upper panel (A) shows the changes in escape latencies in the training phase. The lower panel (B) shows the time spent in the target quadrant (Q4) (in which the platform had been placed during the training phase) in the probe trial (swimming 60 s without platform). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. saline-treated group. All values are means ± S.E.M. ($n = 8–11$).

oxidation of oxymine by O_2^- produced from the xanthine–xanthine-oxidase system (McCord and Fridovich, 1969). Lipid peroxides concentration in the homogenate was assayed by the thiobarbituric acid reaction according to the method of Ohkawa et al. (1979). The levels of lactate and glucose were measured according to the methods of Marbach and Weil (1967) and Morin and Prox (1973), respectively. Protein content was determined by the Coomassie blue protein binding method (Bradford, 1976), using bovine serum albumin as standard.

2.7. Morphology

Three or four rats of each group, chosen randomly from the sham-operated group, the saline-treated group and the huperzine A (0.1 mg/kg)-treated group were decapitated for histopathological observation after the behavior experiment. One hemisphere of each rat was routinely processed and embedded in paraffin. Coronal sections were stained with hematoxylin and eosin.

2.8. Statistics

Group differences in the escape latency in the Morris water maze training task were analyzed using two-way analysis of variance (ANOVA) with repeated measures. One-way ANOVA followed by the Duncan multiple group comparison was used to analyze group differences of the data collected during probe trials. The data from the biochemical studies were compared by Student's *t*-test.

3. Results

3.1. Effects of huperzine A on water maze learning

As shown in Fig. 1A, 4 weeks after surgery, the ligated rats treated with saline exhibited longer escape latencies throughout the training days than did the sham-operated rats [$F(1,9) = 58.366$, $P < 0.001$] in the water maze task. Huperzine A 0.1 mg/kg significantly shortened the escape latencies prolonged by permanent bilateral ligation of the common carotid arteries [$F(1,10) = 11.77$, $P < 0.01$], compared with saline-treated group.

In the probe trials, the time spent in the quadrant (Q4) that had held the hidden platform was used to estimate performance by one-way ANOVA. Fig. 1B indicates that the sham-operated rats and huperzine A 0.1 mg/kg group swam longer(49% and 38% of the time, respectively) in Q4 than did the saline-treated rats (23%).

In order to determine whether the group difference in escape latencies was due to differences in swimming ability, swim speed was calculated for each group, ANOVA showed no difference in swim speed between any two groups.

Table 1
Effects of huperzine A on protein contents in rats chronically hypoperfused after ligation of the bilateral carotid arteries
Rats were killed 40 min after the last administration of huperzine A on day 33 following bilateral ligation of the common carotid arteries. From day 15 to day 33, huperzine A was administered orally twice per day. All values are means ± S.E.M. ($n = 7–11$).

Group	Protein content (mg/g wet wt.)		
	Cortex	Hippocampus	Striatum
Sham-operated	102.9 ± 2.6**	89.7 ± 2.1**	89.0 ± 3.1**
Saline-treated	83.1 ± 3.0	70.8 ± 2.1	63.3 ± 3.2
Huperzine A (0.1 mg/kg)	96.5 ± 1.6**	84.7 ± 2.5**	91.7 ± 2.0**

$^{**}P < 0.01$ vs. saline-treated group.

Table 2
Effects of huperzine A (HupA) on choline acetyltransferase and acetylcholinesterase activities in rats chronically hypoperfused after bilateral ligation of the common carotid arteries
Rats were killed 40 min after the last administration of huperzine A on day 33 following bilateral ligation of the common carotid arteries. From day 15 to day 33, huperzine A was administered orally twice per day. All values are means ± S.E.M. ($n = 7–11$).

Group	Choline acetyltransferase (nmol/h/mg protein)			Acetylcholinesterase (nmol/min/mg protein)		
	Cortex	Hippocampus	Striatum	Cortex	Hippocampus	Striatum
Sham-operated	44.6 ± 1.6	56.4 ± 3.6**	110.3 ± 9.8	71.4 ± 2.7	87.3 ± 2.9	404.6 ± 21.4
Saline-treated	45.9 ± 2.9	22.3 ± 3.8	102.4 ± 10.6	70.9 ± 4.4	93.9 ± 3.0	409.5 ± 18.2
HupA-treated (0.1 mg/kg)	46.0 ± 4.0	61.5 ± 5.0**	107.3 ± 6.2	32.9 ± 2.6**	39.6 ± 1.6**	301.2 ± 9.5**

** $P < 0.01$ vs. saline-treated group.

3.2. Effects of huperzine A on protein content

As shown in Table 1, the protein content per gram wet weight of brain tissues from the saline-treated rats with ligation was significantly decreased in the cortex, hippocampus and striatum (19%, 21%, and 29% lower than sham-operated group, $P < 0.01$) compared with sham-operated rats. Huperzine A elevated the protein content to the non-ischemic levels in the three brain regions ($P < 0.01$ vs. saline-treated group).

3.3. Effects of huperzine A on choline acetyltransferase and acetylcholinesterase activity

Table 2 shows the activity of choline acetyltransferase and acetylcholinesterase in the cortex, hippocampus and striatum. Permanent occlusion of the bilateral common carotid arteries produced a 60% decrease ($P < 0.01$) of choline acetyltransferase activity in the hippocampus area. However, no changes were observed in acetylcholinesterase activity in the cortex, hippocampus and striatum in saline-treated rats with ligation. Huperzine A significantly improved the decrease in choline acetyltransferase activity in the hippocampus ($P < 0.01$), and inhibited acetylcholinesterase activity in the three brain regions.

3.4. Effects of huperzine A on superoxide dismutase activity and lipid peroxides levels

Table 3 shows the improving effects of huperzine A on superoxide dismutase activity and tissue lipid peroxides levels in the cortex, hippocampus and striatum. Superoxide dismutase activity and lipid peroxides levels in the saline-treated rats with ligation were increased significantly ($P < 0.01$) in these brain regions compared with those in the

Table 3
Effects of huperzine A (HupA) on superoxide dismutase activities and lipid peroxides concentration in rats chronically hypoperfused after bilateral ligation of the common carotid arteries
Rats were killed 40 min after the last administration of huperzine A on day 33 following bilateral ligation of the common carotid arteries. From day 15 to day 33, huperzine A was administered orally twice per day. All values are means ± S.E.M. ($n = 7–11$).

Group	Superoxide dismutase (nU/mg protein)			Lipid peroxides (nmol/mg protein)		
	Cortex	Hippocampus	Striatum	Cortex	Hippocampus	Striatum
Sham-operated	40.1 ± 1.5**	46.3 ± 1.9**	46.2 ± 2.6**	2.69 ± 0.07**	2.88 ± 0.10**	3.55 ± 0.17**
Saline-treated	69.0 ± 2.9	74.8 ± 6.0	97.3 ± 5.7	3.70 ± 0.14	4.11 ± 0.17	5.38 ± 0.27
Hup A-treated (0.1 mg/kg)	36.3 ± 1.7**	43.0 ± 2.0**	42.1 ± 1.8**	2.80 ± 0.07**	3.08 ± 0.13**	3.36 ± 0.07**

** $P < 0.01$ vs. saline-treated group.

Table 4
Effects of huperzine A (HupA) on lactate and glucose content in rats chronically hypoperfused after bilateral ligation of the common carotid arteries
Rats were killed 40 min after the last administration of huperzine A on day 33 following bilateral ligation of the common carotid arteries. From day 15 to day 33, huperzine A was administered orally twice per day. All values are means ± S.E.M. ($n = 7–11$).

Group	Lactate (nmol/mg protein)			Glucose (nmol/mg protein)		
	Cortex	Hippocampus	Striatum	Cortex	Hippocampus	Striatum
Sham-operated	410.2 ± 9.2**	546.0 ± 18.8**	492.0 ± 21.0**	415.5 ± 20.2**	483.3 ± 31.7**	450.7 ± 38.1**
Saline-treated	541.1 ± 20.8	729.7 ± 32.2	786.8 ± 41.6	728.8 ± 23.4	820.7 ± 33.4	841.7 ± 38.4
HupA-treated (0.1 mg/kg)	442.6 ± 10.0**	606.2 ± 20.7**	498.1 ± 14.0**	650.7 ± 11.0**	720.2 ± 21.8*	616.4 ± 11.9**

* $P < 0.05$ vs. saline-treated group.
** $P < 0.01$ vs. saline-treated group.

sham-operated group. Huperzine A significantly attenuated the increase in superoxide dismutase activity and lipid peroxides levels induced by chronic hypoperfusion in all three brain regions.

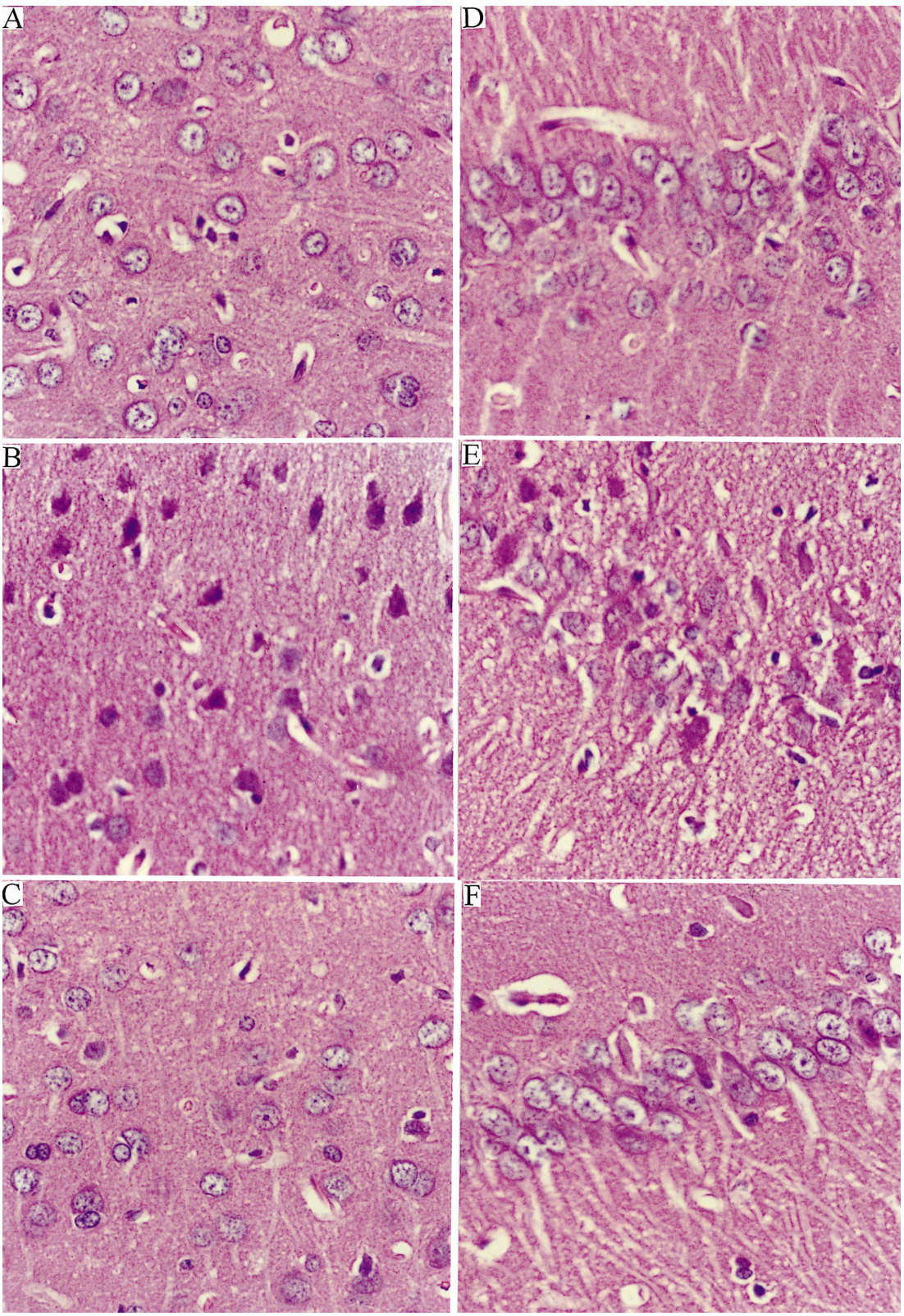

Fig. 2. Photomicrographs of changes in the rat cerebral cortex (A, B and C) and hippocampus CA1 subfield (D, E and F) at 5 weeks after permanent bilateral ligation of the common carotid arteries. (A and D) Sham-operated group; (B and E) saline-treated group; (C and F) huperzine A (0.1 mg/kg)-treated group. Magnification ×40.

3.5. Effects of huperzine A on lactate and glucose content

Table 4 shows the contents of lactate and glucose in the cortex, hippocampus and striatum. In saline-treated rats with ligation, marked increases in lactate and glucose content were observed in the three brain regions ($P < 0.01$). Huperzine A significantly suppressed the chronic hypoperfusion-induced increase in lactate and glucose content in the cortex, hippocampus and striatum.

3.6. Effects of huperzine A on neuronal damage

Fig. 2 shows the typical neuropathological changes observed in the cerebral cortex and hippocampus at 5 weeks after bilateral ligation of the common carotid arteries. Neuronal loss, shrinkage and dark staining of neurons and infarction were observed in the CA1 areas of the hippocampus as well as some infarctions in the cortex in saline-treated rats with ligation. Long-term administration of huperzine A (0.1 mg/kg) attenuated the chronic hypoperfusion-induced neuronal damage.

4. Discussion

Among the animal models that have been developed to study human dementia, the permanently ligated rats are distinct from other kinds of animal models such as drug-, brain lesion-, or transient ischemia-induced amnesia in rodents, because of their progressive and long-lasting cognitive deficits accompanied by progressive neuronal damage. Decreased cerebral blood flow has been observed in 15 brain regions at 1 week after permanent bilateral ligation of the common carotid arteries (Tsuchiya et al., 1993). Of these regions, cortex, hippocampus, and some other regions have been considered to play important roles in learning and memory. In the present behavioral experiment, learning performance in the water maze task was severely impaired in the permanently ligated rats, the impairment having developed for 4 weeks. This result agrees well with previous data showing that ischemia resulted in an increase in the time required to find the hidden platform (Ohta et al., 1997). We found that chronic ischemia induced a decrease in choline acetyltransferase activity in hippocampus and cell death in the CA1 area, both of which had been demonstrated to be correlated to the deficit in spatial learning and memory (Tanaka et al., 1996). These results are also consistent with the notion that the hippocampus and central cholinergic function are critical for intellectual functions such as learning, memory and cognition. In our studies, long-term administration of huperzine A, a potent acetylcholinesterase inhibitor, significantly attenuated the bilateral ligation-induced histological lesions in the brain and improved water maze performance. The dose selected for huperzine A, 0.1 mg/kg, which was proven to produce the maximal improvement (Tang and Han, 1999), with an approximately 33–40% inhibition of acetylcholinesterase activity in the cortex and hippocampus, restored the decreased choline acetyltransferase activity to the sham-operated level. It seems likely that the beneficial effects of huperzine A on the ischemia-induced cognitive deficits were due primarily to its enhancing effects on central cholinergic tone to supplement the impaired ability for acetylcholine synthesis.

The present results, however, do not exclude the possibility that different mechanisms participate in the action of huperzine A on permanent bilateral ligation-induced disruption of water maze performance. It has been well documented that brain ischemia can result in dysfunction of oxidative metabolism, and reduced energy metabolism in the brain is linked to memory impairment (De la Torre et al., 1997), since ATP is a critical energy source for the synthesis, release and inactivation of neurotransmitters as well as for gene expression (transcription and translation) and protein synthesis (Erecinska and Silver, 1989). We observed a marked decrease in protein content and a significant increase in lactate and glucose content in the cortex, hippocampus and striatum in bilaterally ligated rats, similar to the results obtained in previous studies (Takeo et al., 1996). The elevations of tissue lactate and glucose content are considered to be markers of sustained damage to energy production after cerebral ischemia, because the glucose uptake rate decreases and the lactoacidosis increases during the energy-producing process in ischemic rats (Takeo et al., 1996). Treatment with huperzine A preserved the protein content and partly reversed the increase in lactate and glucose content of the brain tissues. Thus, our present findings suggest that the palliative effect of huperzine A on the cognitive deficit is partially due to retardation of the development of sustained ischemia derangement of energy metabolism. As the central cholinergic system is implicated in the regulation of cerebral blood flow (Scremin and Scremin, 1986), an increase in cerebral blood flow may be a factor in the improvement of energy metabolism in ischemic regions, because it enhances washout of lactate accumulated in tissue and the delivery of oxygen to brain regions. However, since there is no evidence that huperzine A is effective to ameliorate the cerebral circulation of the ischemic brain in experimental animals, it would be premature to conclude that there is an enhancement of cerebral blood flow in the ischemic brain by huperzine A.

There is evidence showing that free radicals are capable of mediating neuron degeneration and death, and are possibly involved in the pathogenesis of neuron death in neurodegenerative diseases such as Alzheimer's disease and vascular disease (Markesbery, 1997). In addition to the energy failure caused by chronic hypoperfusion, we found that lipid peroxidation was significantly increased in the three brain regions as a result of free radical generation induced by cerebral ischemia. Meanwhile, the activity of superoxide dismutase, an antioxidant enzyme, was also

elevated in the three brain regions. It is possible that a compensatory rise in antioxidant activity occurs in response to increased free radical generation (Markesbery, 1997). Chronic treatment with huperzine A reversed the abnormality of the free radical system in bilaterally ligated rats. Similar results had been reported for aged rats (Shang et al., 1999) and Alzheimer's disease patients (Xu et al., 1999). Since glutamate is considered to be capable of generating reactive oxygen species (Novelli et al., 1988), and huperzine A has been demonstrated to antagonize the NMDA receptor and to reduce glutamate-induced cytotoxicity (Ved et al., 1997; Wang et al., 1999), it is supposed that huperzine A may exert its effects on the basis of its NMDA receptor antagonism.

Currently, numerous medications such as antioxidants, radical scavengers and calcium antagonists have been approved for the treatment of vascular dementia around the world (Itil et al., 1998). As a closer link between vascular dementia and Alzheimer's disease is gradually being discovered (Pasquier et al., 1998), favorable results have also been achieved with these agents in the treatment of Alzheimer's disease (Kanowski, 1998). From a theoretical point of view, optimal management of risk factors for Alzheimer's disease should include the ability to decrease the incidence rate of vascular dementia (Pasquier et al., 1998). Our findings have substantiated this hypothesis, and demonstrated that multiple mechanisms underlie the therapeutic potential of huperzine A, involving its effects on the cholinergic system, the oxygen free radical system and energy metabolism. Since the pathological changes that occurred after permanent bilateral ligation of the common carotid arteries were quite similar to those observed in patients suffering from multi-infarct dementia, Binswanger's and Alzheimer's disease (Ni et al., 1995), the present findings suggest that huperzine A may be a promising therapeutic agent, not only for Alzheimer's disease, but also for vascular dementia.

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References

Beal, M.F., Hyman, B.T., Koroshetz, W., 1993. Do defects in mitochondrial metabolism underlie the pathology of neurodegenerative disease? Trends Neurosci. 16, 125–131.

Bradford, M.M., 1976. A rapid and sensitive method for the quatitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

De la Torre, J.C., Cada, A., Nelson, N., Davis, G., Sutherland, R.J., Gonzalez-Lima, F., 1997. Reduced cytochrome oxidase and memory dysfunction after chronic brain ischemia in aged rats. Neurosci. Lett. 223, 165–168.

Ellman, G.L., Courtney, K.D., Andre, V. Jr., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activitity. Biochem. Pharmacol. 7, 88–95.

Erecinska, M., Silver, I.A., 1989. ATP and brain function. J. Cereb. Blood Flow Metab. 9, 2–19.

Fonnum, F.J., 1975. A rapid radiochemical method for the determination of the choline acetyltransferase. J. Neurochem. 24, 407–409.

Gottfries, C.G., Blennow, K., Karlsson, I., Wallin, A., 1994. The neurochemistry of vascular dementia. Dementia 5, 163–167.

Itil, T.M., Eralp, E., Ahmed, I., Kunitz, A., Itil, K.Z., 1998. The pharmacological effects of ginkgo biloba, a plant extract, on the brain of dementia patients in comparison with tacrine. Psychopharmacol. Bull. 34, 391–397.

Kanowski, S., 1998. Aging, dementia and calcium metabolism. J. Neural Transm., Suppl. 54, 195–200.

Marbach, E.P., Weil, M.H., 1967. Rapid enzymatic measurement of blood lactate and pyruvate. Clin. Chem. 13, 314–325.

Markesbery, W.R., 1997. Oxidative stress hypothesis in Alzheimer's disease. Free Radical Biol. Med. 23, 134–147.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase, an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.

Morin, L.G., Prox, J., 1973. Single glucose oxidase-peroxidase reagent for two-minute determination of serum glucose. Clin. Chem. 19, 959–962.

Ni, J.W., Matsumoto, K., Li, H.B., Murakami, Y., Watanabe, H., 1995. Neuronal damage and decrease of central acetylcholine level following permanent occlusion bilateral common carotid arteries in rats. Brain Res. 673, 290–296.

Novelli, A., Reilly, J.A., Lysko, P.G., Henneberry, R.C., 1988. Glutamate become neurotoxic via the *N*-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res. 451, 205–212.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Ohta, H., Nishikawa, H., Kimura, H., Anayama, H., Miyamoto, M., 1997. Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. Neuroscience 79, 1039–1050.

Pasquier, F., Leys, D., Scheltens, P., 1998. The influence of coincidental vascular pathology on symptomatology and course of Alzheimer's disease. J. Neural Transm., Suppl. 54, 117–127.

Roman, G.C., Tatemichi, T.K., Erkinjuntti, T., Cummings, J.L., Masdeu, J.C., Garcia, J.H., Amaducci, L., Orgogozo, J.M., Brun, A., Hofman, A., Moody, D.M., O'Brien, M.D., Yamaguchi, T., Grafman, J., Drayer, B.P., Bannett, D.A., Fisher, M., Ogata, J., Kokman, E., Bermejo, F., Wolf, P.A., Bell, M.A., DeCarli, C., Culebras, A., Korezyn, A.D., Bogousslavsky, J., Hartman, A., Scheinberg, P., 1993. Vascular dementia: diagnostic criteria for research studies. Neurology 43, 250–260.

Scremin, O.U., Scremin, A.M.E., 1986. Physostigmine induced reversal of ischemia following acute middle cerebral artery occlusion in the rat. Stroke 17, 1004–1007.

Shang, Y.Z., Ye, J.W., Tang, X.C., 1999. Improving effects of huperzine A on abnormal lipid peroxidation and superoxide dismutase in aged rats. Acta Pharmacol. Sin. 20, 824–828.

Takeo, S., Miyake, K., Tanonaka, K., Takagi, N., Takagi, K., Kishimoto, K., Suzuki, M., Katsuragi, A., Goto, M., Oshikawa, S., 1996. Beneficial effect of nebracetam on energy metabolism after microsphere-induced embolism in rat brain. Arch. Int. Pharmacodyn. 331, 232–245.

Tanaka, K., Ogawa, N., Asanuma, M., Kondo, Y., Nomura, M., 1996. Relationship between cholinergic dysfunction and discrimination

learning disabilities in Wistar rats following chronic cerebral hypoperfusion. Brain Res. 729, 55–65.

Tang, X.C., Han, Y.F., 1999. Pharmacological profile of huperzine A, a novel acetylcholinesterase inhibitor from Chinese herb. CNS Drugs Rev. 5, 281–300.

Tohgi, H., Abe, T., Kimura, M., Saheki, M., Takahashi, S., 1996. Cerebrospinal fluid acetylcholine and choline in vascular dementia of Binswanger and multiple small infarct types as compared with Alzheimer-type dementia. J. Neural Transm. 103, 1211–1220.

Tsuchiya, M., Sako, K., Yura, S., Yonemasu, Y., 1993. Local cerebral glucose utilisation following acute and chronic bilateral carotid artery ligation in Wistar rats: relation to changes in local cerebral blood flow. Exp. Brain Res. 95, 1–7.

Ved, H.S., Koenig, M.L., Dave, J.R., Doctor, B.P., 1997. Huperzing A, a potential therapeutic agent for dementia, reduces neuronal cell death caused by glutamate. NeuroReport 8, 963–968.

Wang, X.D., Zhang, J.M., Yang, H.H., Hu, G.Y., 1999. Modulation of NMDA receptor by huperzine A in rat cerebral cortex. Acta Pharmacol. Sin. 20, 31–35.

Xiao, X.Q., Yang, J.W., Tang, X.C., 1999. Huperzine A protects rat pheochromocytoma cells against hydrogen peroxide-induced injury. Neurosci. Lett. 275, 73–76.

Xu, S.S., Cai, Z.Y., Yang, R.M., Cai, Y.L., Wang, G.Q., Su, X.Q., Zhong, X.S., Cheng, R.Y., Xu, W.A., Li, J.X., Feng, B., 1999. Huperzine A in capsules and tablets for treating patients with Alzheimer's disease. Acta Pharmacol. Sin. 20, 486–490.