Huperzine A attenuates cognitive deficits and hippocampal neuronal damage after transient global ischemia in gerbils

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Received 25 July 2001; received in revised form 4 September 2001; accepted 4 September 2001

Abstract

The protective effects of huperzine A on transient global ischemia in gerbils were investigated. Five min of global ischemia in gerbils results in working memory impairments shown by increased escape latency in a water maze and reduced time spent in the target quadrant. These signs of dysfunction are accompanied by delayed degeneration of pyramidal hippocampal CA1 neurons and by decrease in acetylcholinesterase activity in the hippocampus. Subchronic oral administration of huperzine A (0.1 mg/kg, twice per day for 14 days) after ischemia significantly reduced the memory impairment, reduced neuronal degeneration in the CA1 region, and partially restored hippocampal choline acetyltransferase activity. The ability of huperzine A to attenuate memory deficits and neuronal damage after ischemia might be beneficial in cerebrovascular type dementia. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Huperzine A; Cholinesterase inhibitor; Global ischemia; Morris water maze; Hippocampus; Gerbil

It is well known that delayed neuronal death follows transient cerebral ischemia in selective, vulnerable regions of the brain, especially in the hippocampus. The deficits in learning and memory induced by ischemia show a close correlation with neuronal death in the hippocampal CA1 region [1]. Additionally, the cholinergic system projecting to the hippocampus plays a crucial role in cognitive function, and pre-synaptic cholinergic terminals are sensitive to cerebral ischemia [3,4]. Studies on pathogenic mechanisms have revealed that patients with vascular dementia exhibit cholinergic abnormalities and disturbance of cognitive function similar to those in Alzheimer’s disease [13]. These findings raise the possibility of using cholinergic substances as therapeutic intervention in vascular dementia. It has been reported recently that cholinesterase inhibitor (ChEI) could be protective against ischemia [9].

Huperzine A (HupA), a novel alkaloid isolated from the Chinese herb Huperzia serrata, is a drug with good clinical prospects [11]. Previous studies have shown that, besides inhibiting acetylcholinesterase (AChE), HupA could be broadly neuroprotective [14,17]. This study is designed to explore the action of HupA in cerebral ischemia, and the changes in behavior, morphology and cholinergic indices were observed.

Seventy adult Mongolian gerbils, weighing 75–100 g (purchased from Zhejiang Experimental Animal Center, China) were subjected to transient global ischemia by bilateral occlusion of the common carotid arteries for 5 min under light ether anesthesia [8]. Body temperature was maintained at 37.0 ± 0.5°C using a heating lamp with thermostat. Sham-operated gerbils received the same surgical treatment without occlusion. Oral administration of HupA (0.1 mg/kg) or saline, twice per day, was started at 60 min post-ischemia and terminated on the day of sacrifice (day 14).

The gerbils’ spatial memory performance was evaluated using a Morris water maze [6]. The maze apparatus consisted of a circular pool 150 cm in diameter, 60 cm in depth, filled to a height of 30 cm with water (23 ± 1°C) darkened with Chinese ink. The pool with visual cues on its wall was located in a quiet room and conceptually divided into four equal quadrants (northeast, southeast, southwest and northwest). In the center of the northwest quadrant (Q4), a black platform 10 cm in diameter was submerged approximately 1.5 cm below the water surface. From day 8 after surgery, each gerbil was trained to find the hidden platform for 5 consecutive days with three trials per day. On each trial, the gerbil was lowered gently into the
water facing the pool wall at one of three fixed locations according to a random schedule. In case the gerbil did not succeed within 60 s, it was placed on the platform. At the conclusion of each trial, the gerbils were allowed to remain on the platform for 30 s. Recovery periods of 30 s were allowed between trials. After the final escape training, each gerbil was subjected to a 60 s probe trial in which the platform was removed and the percentage of time spent in Q4 was recorded. Swimming activities were monitored by a video camera linked to a computer-based image analyzer.

For light microscope observation, 4–6 brain samples in each group were formalin-fixed and embedded in paraffin. Consecutive coronal microtome sections of 10-μm thickness taken from a level of 2.0 mm posterior to the bregma were stained with hematoxylin and eosin. For electron microscopy observation, small slices of the hippocampal CA1 region were taken from 3–4 gerbils in each group and placed in 3% glutaraldehyde solution. After post-fixation and dehydration, samples were embedded in epoxy resin and sectioned with an ultramicrotome. Sections (60–70 nm) were stained with uranyl acetate and lead citrate, then examined with a Zeiss EM 902 transmission electron microscope at 80 kV.

For enzyme activity assays, the hippocampus was dissected and homogenized rapidly on ice. Choline acetyltransferase (ChAT) activity was determined using a radiometric method [2]. AChE activity was assayed using a spectrometric method [11]. Protein content was measured by the Coomassie blue protein-binding method.

All results were expressed as means ± SEM. Group differences in the escape latency in the Morris water maze task were analyzed using two-way ANOVA with repeated measures. Group differences in the probe trials and enzyme assays were evaluated with one-way ANOVA followed by Duncan’s multiple range testing, using a computerized statistical package.

As shown in Fig. 1, the mean latency in finding the platform declined progressively during the training period. However, gerbils after ischemia took longer than sham-operated gerbils. This prolongation of latency was markedly shortened by HupA at a dose of 0.1 mg/kg [two-way ANOVA, F(2,8) = 20.860, P < 0.01] (Fig. 1A). In the probe trials, the swimming time spent in Q4 was used to estimate retention performance. Sham-operated group and in ischemia plus HupA group swarm longer in the target quadrant than ischemic group (Fig. 1B). The typical swimming tracks indicated that ischemic gerbils often searched for the platform in an inappropriate way resulting in the longer latency to locate the platform and fewer excursions in the target quadrant (Fig. 1C).

Histological observation of the hippocampus in sham-operated gerbils showed that neurons in the CA1 pyramidal cell layer were clear and moderate-sized with normal ultrastructure. In gerbils with ischemia, the same brain region exhibited significant shrinkage, dark staining, and outright loss of neurons. These neuropathologic signs were suppressed by oral administration of HupA at a dose of

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**Fig. 1.** Effects of huperzine A on Morris water maze performance deficits induced by 5 min cerebral ischemia in gerbils (n = 8–11). Oral administration of huperzine A (0.1 mg/kg, twice per day) for 14 days. (A) Mean daily latencies of escaping from the start point onto the hidden platform. Each gerbil was subjected to three trials per day for 5 consecutive days. (B) Percent of time spent in target quadrant (Q4) in 60 s probe trial (no platform). (C) Typical swim paths (N = north): (a–c) performance on fifth training day. (d–f) performance in probe trial. (a,d) sham-operated group; (b,e) ischemia control group; (c,f) huperzine A-treated ischemia group. Data represent means ± SEM. + P < 0.05, ++ P < 0.01 vs. sham-operated group. *P < 0.01 vs. ischemia control group.
0.1 mg/kg, which reduced the neuronal loss and moderated other morphologic changes. Ultrastructurally, the nucleus appeared irregular in shape with dense chromatin masses on day 14 after ischemia. Within the cytoplasm, extensive vacuolation, dilated organelles and flocculent mitochondrial densities were exhibited. HupA treatment reduced these ultrastructural abnormalities (Fig. 2).

The effects on cholinergic enzymes were complex (Fig. 3). ChAT activity in ischemic gerbils, with or without HupA treatment (0.1 mg/kg for 14 days), was not significantly different from that in sham-operated controls. On the other hand, ChAT activity was significantly higher in ischemic gerbils treated with HupA than in those without drug treatment ($P < 0.05$). This difference can be considered as neurochemical evidence for neuroprotection. It is more difficult to interpret the effects on AChE activity, which decreased significantly on day 14 after ischemia ($P < 0.05$) but was reduced further by HupA ($P < 0.01$) as compared with sham-operated controls.

Our observations in morphology at light and ultrastructural levels in this experiment agree with previous reports [8]. Together with the deficits in water maze performance, these findings support the view that ischemic damage in the hippocampus results in profound impairment of spatial memory tasks [1]. It is therefore impressive that ischemic animals treated with HupA performed better than untreated ischemic animals in acquiring and retaining maze performance. It was also striking that subchronic oral administration of HupA reduced ischemic neuronal damage and gave significant ultrastructural protection in the hippocampal CA1 region. Hence, these data provide direct evidence that HupA can confer marked histopathological and behavioral protection against transient global ischemia at an extended survival interval.

In this study, the decreased AChE activity might be due to persistent impairment of protein synthesis and ischemic damage to cholinergic neurons. The contrasting retention of ChAT activity agrees with a previous biochemical study [3], although others have found immunohistochemical evidence for decreased ChAT in CA1 region [4]. Such discrepancies may simply reflect the difficulty in using whole hippocampal homogenates to detect changes that occur in a limited subregion. In any case it is notable that subchronic oral administration of HupA increased ChAT activity, and might have promoted acetylcholine (ACh) synthesis, which may be beneficial in the ischemia-induced cognitive deficits. Since HupA shows no direct effect on ChAT activity in intact rat and in vitro [12], the increased effects on ChAT here are best explained in terms of cholinergic neuroprotection. The above results indicate that enhanced cholinergic function is involved in the ischemic protection conferred by HupA. Further investigation is necessary to elucidate the relationship between them.

HupA is now in clinical trials for the treatment of Alzheimer’s disease, but emerging information suggests a potential role in the therapy of ischemic disorders as well. The present results confirm and extend our latest investigation demonstrating beneficial effects of HupA in oxygen-glucose deprivation in PC12 cells [18]. Such findings raise important

![Fig. 2. Photomicrographs (A–C) and electron micrographs (D–F) of the hippocampal CA1 region in gerbils after 5 min global ischemia. Oral administration of huperzine A (0.1 mg/kg, twice per day) for 14 days. (A,D) sham-operated group; (B,E) ischemia control group; (C,F) huperzine A-treated ischemia group. Scale bar = 50 μm (A–C) and 2 μm (D–F).](image)
questions about the underlying mechanisms. HupA has been demonstrated to reduce glutamate-induced cytotoxicity by antagonizing cerebral NMDA receptors [16] and this antagonism is one plausible explanation for neuroprotection. Several lines of evidence have shown that HupA reverses abnormalities of the free radical system [10,17], so a similar action could be another potential explanation. A third mechanism to consider is anti-apoptosis. Apoptosis occurs in the delayed neuronal death following transient global ischemia and HupA has been found recently to have anti-apoptotic effects [15]. Finally, we should consider the enhanced cholinergic function resulting from AChE inhibition, since ACh can potentiate the effects of nerve growth factor in vivo, whose protective actions against ischemic insult are confirmed [5].

Currently, links between vascular dementia and Alzheimer’s disease are becoming increasingly apparent. From a theoretical point of view, optimal management of risk factors for Alzheimer’s disease should include the ability to decrease the incidence and severity of vascular dementia [7]. This hypothesis has been substantiated in this study. Our findings suggested that hupA has therapeutic and neurotrophic effects in cerebral ischemia stemming from multiple mechanisms including cholinergic function. We propose that HupA is not only a promising therapeutic agent for Alzheimer’s disease, but also might be beneficial in cerebrovascular type dementia.

This work is supported by the National Natural Science Foundation of China (3001161954). The authors are grateful to Professor Stephen Brimijoin (Mayo Clinic, USA) for his valuable comments on this manuscript and to Mr Tie Feng Zhang and Ms Hong Ying Shan for their technical help on electron microscope.