ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9): 824 - 828
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Improving effects of huperzine A on abnormal lipid peroxidation and superoxide dismutase in aged rats

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KEY WORDS free radicals; aging; lipid peroxidation; malondialdehyde; superoxide dismutase; hippocampus; cerebral cortex; huperzine A

ABSTRACT

AIM: To study the effects of huperzine A on lipid peroxidation and superoxide dismutase (SOD) in hippocampus, cerebral cortex, and serum of aged rats. **METHODS**: The level of lipid peroxidation was determined by thiobarbituric acid reactive substance method and represented as the concentration of malondialdehyde (MDA) in wet tissue. The activity of SOD was determined by xanthine-xanthine oxidase method and represented as the nitrite unit per gram protein in wet tissue. **RESULTS**: The levels of MDA and the manganese-SOD (Mn-SOD) activities in hippocampus, cerebral cortex, and serum of aged male rats were 2.3 - 2.8 times and 1.8 - 2.8 times, respectively, higher than those of adult male rats. Huperzine A (0.05 mg/kg, ig) lowered markedly the levels of MDA and the activities of Mn-SOD in aged male rats following 7 - 14 d consecutive administrations. The MDA levels in hippocampus, cerebral cortex, and serum decreased 44.7 - 52.8 % (7 d) and 52.6 - 54.7 % (14 d). The Mn-SOD activities in hippocampus, cerebral cortex, and serum lowered 25.0 - 57.6 % (7 d) and 56.0 - 74.2 % (14 d). In adult rats, no marked change was found after 7 - 14 d consecutive administrations of huperzine A at a dose of 0.05 mg/kg. **CONCLUSION**: Huperzine A improved the abnormal free radicals in aged rats.

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Accepted 1999-06-22

INTRODUCTION

Increased free radicals mediated lipid peroxidation and alteration of superoxide dismutase (SOD) in some brain regions might respond for further free radical generation and neuron death in Alzheimer's disease (AD). The central nervous system was easily damaged by free radicals as results of the brain's high oxygen consumption rate, its abundant lipid content, and the relative paucity of antioxidant enzymes compared with other tissues. The appealing feature of the oxidative stress hypothesis for neurodegenerative disease was that accumulative oxidative damage over time could account for the late life onset and the slowly progress nature of these diseases[1]. Free radical hypothesis suggested that free radical system might play an important role in aging process[2]. Free radicals were highly reactive and cytotoxic due to their strong oxidizing properties. In aging, the possible consequences of prolonged exposure to accumulation of reactive free radicals were alteration in lipid peroxidation and antioxidative system, and furthermore the progressive defects in protection against free radical reactions allow tissues, especially the central nervous system, to be vulnerable to damage and finally induce some neurodegenerative diseases[1,3]. There was evidence that free radicals played a role in neurodegenerative diseases such as AD[1].

Huperzine A (Hup-A), a *Lycopodium* alkaloid isolated from the Chinese herb *Huperzia serrata* (Thunb) Trev, was a highly selective and reversible acetylcholinesterase (AChE) inhibitor[4]. Hup-A improved learning and memory in various animal models mainly owing to its AChE inhibition[4-6]. Cholinesterase inhibitor (ChEI) has a neuroprotective effect[7-9]. Hup-A could inhibit NMDA-dependent glutamate-induced cytotoxicity[7,8] and other ChEI such as tacrine inhibited β-amyloid-induced cytotoxicity[9].

These two systems may be involved in the generation of free radicals[10]. So far there is no report concerning the effects of ChEI on free radical system in central nervous system. The aim of this study was to investigate the alterations of lipid peroxidation and SOD activity in aged rats after subacute administration of Hup-A.

MATERIALS AND METHODS

Rats Sprague-Dawley adult male rats (2 months) and aged male rats (22 - 24 months) were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate No 005).

Materials *l*-Hup-A (purity >98 %) was provided by Department of Phytochemistry in this Institute. The reagent kits for measurement of the level of malondialdehyde (MDA) and the activity of manganese-superoxide dismutase (Mn-SOD) were purchased from Nanjing Institute of Jiancheng Biological Engineering. Other reagents were AR grade.

Preparation of samples Rats were decapitated 30 min after last ig Hup-A. Control rats were given saline. The brain was rapidly dissected on ice. The hippocampus and cerebral cortex were homogenzined in cold Tris-HCl buffer to obtain 10 % homogenates used to assay MDA, and 1 % homogenates used for determination of the activity of Mn-SOD. Blood was collected in heparinized tubes and centrifuged at 3500 × *g* for 15 min to obtain serum for determination of MDA directly. The serum was diluted with cold Tris-HCl buffer (1∶10, vol/vol) for determination of Mn-SOD activity. The whole process of measurement was in ice-cold environment before incubating.

MDA assay Since MDA was the final product of lipid peroxidation, the concentration of MDA could reflect the level of lipid peroxidation in tissue indirectly. MDA could be combined with thiobarbituric acid to produce a red substance. The red substance had maximal absorbance (A) at $\lambda = 532$ nm. The measurement was according to the instruction of reagent kits. The levels of MDA were calculated as follows:

MDA level in serum = $(A_1 - A_2) \div (A_3 - A_4) \times C$ μmol/L serum

MDA level in wet tissue = $(A_1 - A_2) \div (A_3 - A_4) \times C \times D \div 100$ μmol/g wet tissue. Where A_1 and A_2 were absorbance of sample and sample blank, respectively. A_3 and A_4 were absorbance of standard and standard blank, respectively. C was the standard concentration and D was the dilute times of sample.

Mn-SOD activity assay The activity of SOD was capable of scavenging free radicals in tissue. The xanthine-xanthine oxidase system produced superoxygen free radicals. Superoxygen free radicals could oxidize hydroxy amine to produce nitrite, which reacted with developer to produce a purple substance. The purple substance had maximal absorbance (A) at $\lambda = 550$ nm. The measurement was according to the instruction of reagent kits. The SOD activity was calculated as follows:

SOD activity in tissue protein = $(A_1 - A_2) \div A_1 \div 50\ \% \times D \div C$ kU/g protein. Where A_1 and A_2 were absorbance of control and sample, respectively. D was the dilute times of sample in reactive system, and C was the concentration of tissue protein.

Protein assay Protein amount was measured by the Coomassie blue protein-binding method using bovine serum albumin as standard[11].

Statistical analysis Data were expressed as $x \pm s$ and compared with t-test.

RESULTS

Effects of Hup-A on lipid peroxidation in aged and adult rats The levels of MDA in hippocampus, cerebral cortex, and serum of aged rats were 2.8, 2.3, and 2.5 times higher than those of adult rats ($P < 0.01$, Tab 1).

Tab 1. Comparison of MDA level and Mn-SOD activity in adult and aged male rats. $n = 10$ rats. $\bar{x} \pm s$. Data were expressed as μmol/g wet tissue or μmol/L serum (MDA) and kU/g protein (SOD). $^cP < 0.01$ *vs* adult group.

Rats		Hippocampus	Cerebral cortex	Serum
Adult	MDA	1.9 ± 0.10	2.3 ± 0.07	6.1 ± 0.4
	Mn-SOD	2.8 ± 0.27	2.8 ± 0.32	0.24 ± 0.03
Aged	MDA	5.3 ± 0.23^c	5.4 ± 0.19^c	15.2 ± 1.71^c
	Mn-SOD	6.0 ± 0.4^c	5.0 ± 0.32^c	0.66 ± 0.08^c

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Consecutive administrations of Hup-A (0.05 mg/kg, ig, bid) lowered the MDA levels in aged rats ($P < 0.01$, Tab 2). The MDA levels decreased 52.8 % (hippocampus), 50.0 % (cerebral cortex), and 44.7 % (serum) for 7 d and 54.7 % (hippocampus), 53.7 % (cerebral cortex), and 52.6 % (serum) for 14 d. In adult rats, no marked change of MDA levels were found after administration of Hup-A (0.05 mg/kg, ig, bid) for 7 d and 14 d ($P > 0.05$, Tab 3).

Effects of Hup-A on Mn-SOD activity in aged and adult rats The activities of Mn-SOD in hippocampus, cerebral cortex, and serum of aged rats were 2.1, 1.8, and 2.8 folds higher than those of adult rats ($P < 0.01$, Tab 1). Hup-A (0.05 mg/kg, ig, bid) decreased Mn-SOD activities following administration in aged rats ($P < 0.05$, $P < 0.01$, Tab 2). Compared with saline group, the Mn-SOD activities decreased 25.0 % (hippocampus), 30.0 % (cerebral cortex), and 57.6 % (serum) for 7 d and 58.3 % (hipocampus), 56.0 % (cerebral cortex), and 74.2 % (serum) for 14 d. Administration of Hup-A (0.05 mg/kg, ig, bid) for 7 d and 14 d did not yield much change in Mn-SOD in adult rats ($P > 0.05$, Tab 3).

DISCUSSION

The present studies showed that the concentration of MDA markedly increased in hippocampus, cerebral cortex, and serum of male aged rats compared with adult control. Our finding was consistent with previous reports[12-15]. The features of brain were liable to be peroxidated by free radicals. It contained a large amount of phospholipids that were rich in polyunsaturated fatty acids that were vulnerable to be peroxidated by free radicals, especially by singlet oxygen and hydroxyl radical. Lipid peroxidate readily decomposed to liberate carbonyl fragments, the most prominent being MDA that was highly reactive and responsible for cytotoxic effects and neuron death[1,16]. Therefore the level of MDA was one way of the extent of brain damage due to free radicals in aging[1,16], and furthermore affected the advanced function of brain such as learning and memory[17].

SOD was thought to be one of the major enzymes that protect against tissue damage caused by the potentially cytotoxic reactivities related to aging of the organism. The reported abnormal alteration in SOD activities with age may further accelerate the aging

Tab 2. Effects of oral huperzine A on MDA level and Mn-SOD activity in aged male rats.
$n = 10$ rats. $\bar{x} \pm s$. Data were expressed as μmol/g wet tissue or μmol/L serum (MDA) and kU/g protein (SOD). $^bP < 0.05$, $^cP < 0.01$, *vs* saline control.

Group	Dose/mg·kg^{-1} bid × d	Hippocampus		Cerebral cortex		Serum	
		MDA	Mn-SOD	MDA	Mn-SOD	MDA	Mn-SOD
Saline	/	5.3 ± 0.23	6.0 ± 0.4	5.4 ± 0.19	5.0 ± 0.32	15.2 ± 1.71	0.66 ± 0.08
Huperzine A	0.05 × 7	2.5 ± 0.12^c	4.5 ± 0.35^b	2.7 ± 0.18^c	3.5 ± 0.52^b	8.4 ± 0.44^c	0.28 ± 0.03^c
	0.05 × 14	2.4 ± 0.27^c	2.5 ± 0.27^c	2.5 ± 0.13^c	2.2 ± 0.11^c	7.2 ± 0.39^c	0.17 ± 0.02^c

Tab 3. Effects of oral huperzine A on MDA level and Mn-SOD activity in adult male rats.
$n = 6$ rats. $\bar{x} \pm s$. Data were expressed as μmol/g wet tissue or μmol/L serum (MDA) and kU/g protein (SOD). $^aP > 0.05$, *vs* saline control.

Group	Dose/mg·kg^{-1} bid × d	Hippocampus		Cerebral cortex		Serum	
		MDA	Mn-SOD	MDA	Mn-SOD	MDA	Mn-SOD
Saline	/	2.3 ± 0.11	3.1 ± 0.78	3.0 ± 0.32	2.9 ± 0.61	8.9 ± 1.19	0.26 ± 0.03
Huperzine A	0.05 × 7	2.4 ± 0.35^a	3.0 ± 0.36^a	3.3 ± 0.28^a	3.0 ± 0.31^a	8.9 ± 0.49^a	0.27 ± 0.04^a
	0.05 × 14	2.5 ± 0.27^a	2.5 ± 0.41^a	3.0 ± 0.09^a	2.8 ± 0.34^a	8.4 ± 0.96^a	0.25 ± 0.04^a

process. Some findings even suggested a causal relationship between antioxidant enzyme activities including SOD and life span of animal species[1]. We also found that the Mn-SOD activities in hippocampus, cerebral cortex, and serum of male aged rats increased markedly which were consistent with many studies[16,18], but different from some other results[19,20]. These discrepancies might be due to differences in methodologies for tissue preparation and enzyme determination or experimental model[13]. The precise mechanism of high SOD activity in aging animal was not clear. The possible explanation was that SOD gene irregular expression may occur as a feedback to the accumulation of free radicals with aging[21-23].

Our studies showed that consecutive administration of Hup-A could markedly decrease the level of MDA and Mn-SOD activity in aged rats, but had no effect on the normal free radical system in adult rats. It might suggest that the effects of Hup-A were specific to abnormal change of free radical system. Hup-A exerted these effects possibly through two different ways. One way was that it antagonized NMDA receptor and reduced NMDA-dependent calcium influx and cytotoxicity[7,8]. The another way might be related to the activation of nicotinic receptor through increasing ACh level at synapse[24], which might inhibit glutamate-or-amyloid-induced cytotoxity[8,24]. Both of these were involved in generation of free radicals[9,10]. The improving effects of Hup-A on free radical system were also observed in clinical trials. Hup-A could ameliorate the abnormal increasing of MDA level and decreasing of SOD activity in erythrocytes and plasma of patients with AD[25]. These findings suggested that the neuroprotection against free radicals-induced cytotoxicity by Hup-A may slow down or block the pathogenesis process in AD.

ACKNOWLEDGMENTS To Prof ZHU Da-Yuan for preparation of huperzine A and Ms WANG Hong for technical assistance in experiment.

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ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Sep; 20 (9)
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石杉碱甲对老年大鼠异常的脂质过氧化和超氧化物歧化酶的改善作用

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关键词 自由基；衰老；脂质过氧化作用；丙二醛；超氧化物歧化酶；海马；大脑皮质；石杉碱甲

目的：研究石杉碱甲对老年大鼠海马、皮层和血清的脂质过氧化和超氧化物歧化酶的作用. **方法**：硫代巴比妥酸法测定组织中 MDA 水平；黄嘌呤氧化酶法测定组织中超氧化物歧化酶的活性. **结果**：雄性老年大鼠海马、皮层和血清中 MDA 水平和 Mn-SOD 活性明显高于成年大鼠. 石杉碱甲明显降低雄性老年大鼠海马、皮层和血清中 MDA 水平和 SOD 活性，而对成年大鼠的这两项指标无明显影响. **结论**：石杉碱甲能明显改善衰老导致的自由基系统的异常变化，这种神经保护作用可能有益于 AD 的治疗.

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