Effect of polygonum multiflorum on the fluidity of the mitochondria membrane and activity of COX in the hippocampus of rats with Aβ 1-40-induced Alzheimer's disease

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Abstract: **Objective** To explore the effect of polygonum multiflorum on the fluidity of mitochondria membrane and activity of cytochrome oxidase (COX) in Alzheimer's disease (AD) model rats. **Methods** Forty-five SD rats were randomly divided into 3 groups: an AD model group, a control group, and a treatment group (n = 15). AD model was established by injecting beta-amyloid protein (Aβ) 1-40 into the hippocampus of rats. The learning and memory abilities of rats were tested with the Y-electrical maze. The coefficient of viscosity of the hippocampal mitochondria membrane was determined by a spectrofluorometer, and the activity of COX was measured by an ultraviolet spectrophotometer. **Results** Compared with the control group, the learning and memory ability of the AD model group was significantly lower ($P<0.01$), while the coefficient of viscosity of the hippocampal mitochondria membrane of the AD model group rats was significantly higher ($P<0.01$), and COX activity was lower ($P<0.01$). Compared with the AD model group rats, the coefficient of viscosity of the hippocampal mitochondria membrane of the treatment group was significantly lower ($P<0.05$), and COX activity was significantly improved ($P<0.05$). **Conclusion** Polygonum multiflorum could improve the fluidity of mitochondria membrane and the activity of mitochondrial COX in the model of Alzheimer's disease.

Key words: polygonum multiflorum; Alzheimer's disease; beta-amyloid protein; fluidity of mitochondria membrane; cytochrome oxidase

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Biography HOU De-ren (1966-), M. D., associate Prof., mainly engaged in the research of Alzheimer's disease.

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何首乌对 Aβ1-40 诱导的 AD 大鼠海马线粒体膜流动性及 COX 活性的影响

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[摘要] 目的:探讨何首乌对 AD 模型大鼠线粒体膜流动性和 COX 活性的影响。方法:45 只大鼠随机分为对照组、模型组和治疗组($n=15$)。大鼠海马内注射 Aβ1-40 建立 AD 模型。Y-型迷宫实验检测 AD 模型大鼠的学习记忆能力。运用荧光光度计和紫外分光光度仪分别检测海马线粒体膜的黏滞系数和细胞色素氧化酶(COX)活性。结果:与对照组比较,模型组大鼠学习记忆能力的下降($P<0.01$),模型组大鼠海马线粒体膜的黏滞系数增高($P<0.01$),细胞色素氧化酶活性明显减低($P<0.01$);与 AD 模型组比较,治疗组大鼠线粒体膜的黏滞系数降低($P<0.05$),细胞色素氧化酶活性明显增高($P<0.05$)。结论:何首乌可改善 AD 模型大鼠海马线粒体膜流动性,提高其细胞色素氧化酶活性。

[关键词] 何首乌; 阿尔茨海默病; 淀粉样蛋白; 线粒体膜流动性; 细胞色素氧化酶
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Alzheimer's disease (AD) is a neurodegenerative disease with unknown causes and is related to aging. Recent studies have shown that oxidative damage of the neuronal mitochondria plays important roles in the genesis and development of AD[1-2]. Mitochondria are not only the main site of energy metabolism of cells, but also the principal organelle generating oxygen free radicals. Suitable fluidity of the mitochondria membrane plays vital roles in the maintenance of mitochondrial functions. Cytochrome oxidase (COX) is a rate-limiting enzyme and is the terminal enzyme of the mitochondrial electron transport chain and thus plays key roles in regulating energy metabolism in cells. Our previous studies have shown that polygonum multiflorum thunb can improve the learning and memory abilities, and the mechanism is probably related to its antioxidant property and reducing the damage of cell membrane[3]. In this study, we further explored the effect of polygonum multiflorum thunb on the fluidity of the mitochondria membrane and activity of COX in rats with AD.

1 MATERIALS AND METHODS

1.1 Reagent and laboratory apparatus Beta-amyloid protein 1-40 (Aβ 1-40) was purchased from the Sigma Company in USA, diluted with physiological saline (10 g/L), incubated at 37 ℃ to form an aggregation, and then stored at 4 ℃. The SN-3 stereotaxic apparatus was made in Japan, and the Y-electrical maze was made as described elsewhere[4].

1.2 Animals and groups Sprague Dawley (SD) rats, of random gender, weighing 220 ~ 250 g, were provided by the Laboratory Animal Center of the Central South University. We housed them in the animal room of the laboratory for 3 days before the experiment. Learning ability and memory were tested by the Y-electrical maze as follows: We considered a correct reaction to be an escape from the primary region to a safe one directly after electric stimulation. We measured the times as a marker of learning and memory. Smaller values corresponded

with better learning ability and memory. We placed the rats in the Y-type electrical maze for 5 minutes prior to testing to allow them to become familiar with the internal construction comprising 3 paths, and then began the test from one direction, which was selected randomly. An electric current (30 ~ 70 voltage, 0.5 ~ 0.7 mA) was applied in each test. There was a resting interval of 30 seconds between every two tests, and 5 minutes between every 10 tests. The learning ability and memory of rats were considered if the rats escaped directly to the safe region 9 times in 10 sequential tests. Rats that did not correctly choose the safe region 9 times in any 10 sequential tests of 30 tests were excluded from the study. We screened 45 rats (not including rats excluded from the study) and randomly divided them into 3 groups: a control group, an AD group, and a treatment group.

1.3 Establishment of AD model Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.35 mg/kg) and fixed in the stereotactic frame. The skin was incised according to the midline to reveal the front fontanelle. The target coordinate of the right hippocampus was 3.0 mm posterior to the front fontanelle, 2.0 mm right to the midline, and 2.9 mm ventral to the surface of the cortex, as described elsewhere[5]. The skulls of all rats were drilled and Aβ 1-40 was slowly injected (over a 5-minute period of time) into the coordinate point of rats in the AD group and the treatment group, while physiological saline was injected to the control group in the same manner. The needle was slowly removed after being kept in place for another 5 minutes, and then the incision was carefully sutured. Aseptic physiological saline (1 mL) was administered twice daily into the stomachs of rats in the control and AD groups, while rats in the treatment group were given polygonum multiflorum thunb (1 mL) dissolved in saline.

1.4 Preparation of the hippocampal mitochondria The rats were sacrificed with intraperitoneal 10% chloral hydrate (3.5 mL/kg) 40 days after the establishment of AD. The hippocampus on both sides was removed and placed on ice, and then homogenized at 4 ℃ after the addition of 5 mL sucrose (0.25 mmol/L). The homogenate was placed in 0.34 mmol/L sucrose, centrifuged at 700 r/min for 10 minutes, and the resulting supernatant was centrifuged at 5 000 r/min for 10 minutes. The precipitate was added to 2 mL sucrose (0.25 mmol/L) and centrifuged at 5 000 r/min for 20 minutes. The resultant precipitate contained the mitochondria and was centrifuged again and immersed in sucrose (0.25 mmol/L).

1.5 Determination of the fluidity of mitochondria membrane We added 2 mL DPH liquid (2×10^{-6} mol/L) to a 2 mL suspension of the mitochondria, incubated the samples at 25 ℃ for 30 minutes after mixing, and then centrifuged the samples for 10 minutes at 5 000 r/min at 4 ℃. The supernatant was removed and 3.5 mL PBS (0.1 mol/L) was added to the precipitate.

AHITACHI F-4000 Fluorescence Spectrophotometer (excitation wavelength: 362 nm; emission wavelength: 432 nm) was used to measure: (1) IVV — the fluorescence intensity when both the optical axes of the polarizer and the analyzer were vertical; (2) IVH — the fluorescence intensity when the optical axis was vertical and the analyzer was horizontal; (3) IHV — the fluorescence intensity when the optical axis was horizontal and the analyzer was vertical, and (4) IHH — the fluorescence intensity when both the optical axis and the analyzer were vertical. η, the representation of the value of coefficient of viscosity of the mitochondria membrane, was calculated according to the formula:

$G = \mathrm{IHV}/\mathrm{IHH}$

$P = (\mathrm{IVV} - G\ \mathrm{IVH})/(\mathrm{IVV} + G\ \mathrm{IVH})$

$\eta = 2P/(0.46 - P)$.

The higher the value of η, the lower the fluidity of the mitochondria membrane.

1.6 Determination of COX activity The activity of COX was determined as previously described[6-7]. In brief, 0.5 mL KH_2PO_4 (pH 7.4,

200 mmol/L) was mixed with 0.375 mL H_2O_2, 25 μL Triton X-100 (volume/volume: 2%), 67 μL mitochondria (20 ~ 30 μg), 33 μL cytochrome C (20 mg/mL, deoxidized by Vitamin C at $A550/A565 > 12$ before use). An ultraviolet spectrophotometer was used to determine the absorption value of COX at 550 nm.

1.7 Statistical analysis Data are expressed as $\bar{x} \pm s$ and were analyzed by SPSS 11.0. Comparisons between groups were performed by one-way analysis of variance (ANOVA) and evaluated by the least significant difference test (*LSD-t*). Data were considered significant if the $P < 0.05$.

2 RESULTS

2.1 Learning and memory abilities of the AD model rats Before the induction of AD, there was no significant difference in learning and memory abilities between the groups ($F = 0.311$, $P > 0.05$). As expected, after induction of AD, the learning and memory abilities were significant different between the groups ($F = 28.01$, $P < 0.05$). The difference was highly significant between the AD model group and the control group ($t = 12.60$, $P < 0.01$), which showed that injection of Aβ 1-40 into the hippocampus could simulate the pathology of AD, as reflected by the regressed learning and memory abilities of these rats. In addition, there was a significant difference between the treatment group and the AD model group ($P < 0.05$), which demonstrated that polygonum multiflorum could attenuate the affects on learning and memory abilities associated with AD (Tab. 1).

Tab. 1 Comparison of learning and memory abilities among the 3 groups ($\bar{x} \pm s$, $n = 15$)

Group	Before AD induction	After AD induction
Control	13.4 ± 3.3	13.1 ± 2.9
Model	12.9 ± 2.4	25.2 ± 3.3**
Treatment	12.0 ± 2.6	18.6 ± 2.8#

Compared with the control group, ** $P < 0.01$; compared with the model group, #$P < 0.05$.

2.2 Coefficient of viscosity of mitochondria membrane in the hippocampus Overall, there was a significant difference in membrane viscosity between groups ($F = 6.397$, $P < 0.05$). This was partly due to the difference between the model group and the control group ($P < 0.01$), as the viscosity of the mitochondria membrane in hippocampus was greater in the AD group. In addition, the coefficient of viscosity of mitochondria membrane was significantly lower in the treatment group compared with the model group ($P < 0.05$) (Tab. 2).

Tab. 2 Effect of polygonum multiflorum on the fluidity of mitochondria membrane ($\bar{x} \pm s$, $n = 15$)

Group	Viscous coefficient of mitochondrial membrane(η)
Control group	1.4951 ± 0.4351
Model group	3.3055 ± 1.3867**
Treatment group	1.8228 ± 0.7113#

Compared with the control group, ** $P < 0.01$; compared with the model group, #$P < 0.05$.

2.3 Mitochondrial COX activity There was significant difference in the absorbance value between groups ($P < 0.05$). Compared with the control group, the absorbance value was significantly lower in the model group ($P < 0.01$). Compared with the model group, the absorbance value was significantly higher in the treatment group ($P < 0.05$) (Tab. 3).

Tab. 3 Effect of polygonum multiflorum on the activity of COX in the mitochondria of the hippocampus ($\bar{x} \pm s$, $n = 15$)

Group	COX activity
Control group	0.33468 ± 0.08106
Model group	0.21794 ± 0.05322**
Treatment group	0.29616 ± 0.02266#

Compared with the control group, ** $P < 0.01$; compared with the model group, #$P < 0.05$.

3 Discussion

Previous studies have indicated that suitable fluidity of the biomembrane is essential for the maintenance of physiological function of its receptors, ion channels and calcium pumps. The mitochondrial membrane comprises a large proportion of unsaturated fatty acid and so is vulnerable to oxygen free radical. As the membrane plays important roles in regulating glycometabolism and biological oxidation, any changes in structure and/or fluidity, will interfere with glycometabolism and biological oxidation. Previous studies have found that Aβ may alter the structure and function of the mitochondrial membrane with changes in membrane viscosity, deprivation of energy, reactive oxygen species production, and cytochrome C release[8]. It has been found that the metabolism of tissue in the brain of AD patients decreases, which indicates that damage to the structure and impaired function of the mitochondria may play vital roles in the development of AD[9-10]. In our present study, we found that the coefficient of viscosity of hippocampal mitochondrial was significantly greater in the model group. This will inevitably lead to decrease mitochondrial membrane fluidity and interfere with its normal function. Compared with the model group, the coefficient of viscosity in the treatment group was significantly lower, which indicates that polygonum multiflorum thunb may help protect the mitochondrial membrane fluidity and lessen damage to the structure and function of the mitochondria. We have found in our previous studies that polygonum multiflorum thunb may reduce the content of malonaldehyde of hippocampus in rats and increase the activity of superoxide dismutase in the AD model, which indicates that polygonum multiflorum thunb may help protect the brain from oxidative damage[3]. Therefore, we suggest that polygonum multiflorum thunb maintains the fluidity of mitochondria membrane and protects it from being damaged through its role as an antioxidase.

COX is the terminal enzyme and the rate-limiting enzyme in the mitochondrial electron transport chain, and thus plays key roles in the regulation of energy metabolism. The maintenance of normal physiological function, metabolism and structural integrity of neurons relies on the energy obtained from energy-rich phosphate compounds. Under normal conditions, there is only a small reserve of high-energy phosphate and carbohydrates in neural tissue, so the brain is extremely sensitive to change in energy metabolism. In this sense, we can conclude that the level of energy metabolism in the brain tissue indirectly reacts to the activity level of neurons. Therefore, the function of neurons is intimately related to the activity of COX, which is a biomarker for the degree of neuronal endogenous metabolism. In the present study, COX activity in the AD rats was significantly lower compared with the control group, while COX activity in the treatment group was significantly higher than that in the AD model group. These results indicate that polygonum multiflorum thunb may increase the activity of mitochondrial COX. It has been reported that the mammalian COX enzyme consists of 13 subunits. The longest 3 are COX I, II, and III, which comprise the main structure of the active core of the enzyme, and are all encoded by mtDNA. Damage to the structure and function may lead to the change of COX activity, as has been shown in previous studies[11]. Mitochondria are not only the main site of metabolism, but also an important cell organelle where oxygen free radicals are produced. Therefore, the internal structure of mitochondria, such as mtDNA, is the primary target of the oxygen free radicals[12]. We conclude that polygonum multiflorum thumb may play a role in maintaining the function of mitochondria by reducing the damage of mtDNA and by maintaining COX activity. Further studies are needed to better elucidate the molecular mechanism involving mtDNA.

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