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Protective effect of taurine against Aβ$_{1-40}$-induced toxicity

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Abstract: **In this study, we evaluated the effects of taurine against Aβ$_{1-40}$-induced neurotoxicity in vivo and in vitro experiments. Rats received an intracerebroventricular fusion of Aβ$_{1-40}$ and were given taurine by intragastric administration. Then behavioral performance was assessed, which revealed that 3.2, 4.79mmol/kg taurine treatment promoted positive cognitive effects in Aβ$_{1-40}$-induced rats. Further, an Aβ$_{1-40}$-induced toxicity in human neuroblastoma SH-SY5Y cell model was used for investigating the mechanisms of taurine. Exposure of SH-SY5Y cells to Aβ$_{1-40}$ caused cell apoptosis, reduction in cell viability and mitochondrial membrane potential ($^{\triangle}\Psi$ m). Pretreatment of the cells with 8, 20mmol/L taurine before Aβ$_{1-40}$ exposure significantly attenuated those changes in a dose-dependent manner. These results show that taurine has significant protective effects against Aβ$_{1-40}$-induced toxicity in rats and SH-SY5Y cells, and the action mechanism may be associated with up-regulating mitochondrial membrane potential.**

Keywords: Taurine; β-Amyloid peptide (Aβ) ; Alzheimer's disease

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by global cognitive decline including progressive memory, learning and reasoning impairment. This gradual decline of these abilities corresponds with the presence of senile plaques, neuronal loss and neurofibrillary tangles[1-3]. The pathogenesis of AD , such as Aβ toxicity, Tau hyperphosphorylation, cholinergic disorder, has not been completely clarified. Studies have revealed evidence for a possible and central causative role of Aβ which is implicated with mitochondrial dysfunction in AD.

Taurine is a sulfur containing amino acid, which is in substantial amounts in central nervous system. Taurine has been demonstrated to be involved in many important physiological functions and have neuroprotective properties[4,5]. In AD a decrease in taurine concentration has been provided in the brain of the AD patients. Moreover, it has been indicated that taurine prevents the neurotoxicity of A β and that the protection is related to the activation of $GABA_A$ receptors[6]. As mentioned above, we hypothesized that taurine could be both alleviating symptoms of AD, and protecting the neurons. In order to test our hypothesis, we used the rats model and cell model of AD induced by Aβ$_{1-40,}$ and examined the effects of taurine against Aβ$_{1-40}$-induced toxicity in SD rats and SH-SY5Y cells.

2. Material and methods

2.1. Animals and Cell line

Eighty-four adult male Sprague-Dawley mice (200±20g) were purchased from the Experiment Animal Center of Qingdao Drug Inspection Institute. Animals were acclimatized for 1 week before the onset of the experiments and were allowed free access to food and water. All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Ethics Committee of Qingdao University Medical College.

The SH-SY5Y human neuroblastoma cell line was obtained from Peking Union Medical College.

2.2. Drugs and reagents

Taurine, Aβ$_{1-40}$, Hoechest 33258, 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT) and Rhodamine123 were all purchased from Sigma Chemical Co in the United States, and DMEM medium from HyClone in the United States. The purity of tautine was analyzed by HPLC (>95%). Aβ$_{1-40}$ was dissolved in sterile saline (1mM) and aggregated by incubation at 37 ℃ for 7 days before use. All other chemicals were of analytic grade.

2.3. Instruments and equipments

Morris water maze was made by Institute of Materia Medica of Chinese Academy of Medical Sciences. Stereotaxic instrument was made by Narishige. The ELISA reader was made by America Bio-Rad. Flow cytometer was made by BD Biosciences, San Jose, CA.

2.4. Grouping and Establishment of AD model

SD rats were randomized into 7 groups, with 12 rats in each group: control group, sham group, model group, positive group, taurine group (4.79, 3.2, 1.6mmol/kg). After 7 days breeding, the rats were given normal saline by intragastric administration in control group, sham group and model group. The rats of positive drug group were given Hup A in the dose of 0.66μmol/kg. The rats of taurine groups were given taurine by intragastric administration in its respective dose (4.79, 3.2, 1.6mmol/kg).

On the 11th day, SD rats were anesthetized with Chloral hydrate 10% (3.5ml/kg) and mounted in a stereotaxic apparatus. Intracerebroventricular administration of Aβ was carried out with the procedure described previously[7]. The aggregated of $A\beta_{1-40}$ (5μl) or sterile saline was administered at the speed of 1μl/min into the right lateral ventricle with the following coordinates: -3.0mm anteroposterior, -2.2mm later to bregma, and -2.8mm ventral to the skull surface[8]. The microsyringe was held for 5 min for adequate dispersion of $A\beta_{1-40}$. After surgery, rats would be given Huperzine A, taurine or normal saline by intragastric administration continuously until the end of the experiment.

2.5. Behavioral study

Learning and memory abilities of rats were assessed by the Morris water maze (MWM) test, which was consisted of a round pool and automatical analysis systems. The pool, which was filled with water at a temperature of 25.0±1℃ to avoid hypothermia as described previously[9]with minor modifications, was divided equally into four quadrants. The platform (10cm in diameter), which is submerged approximately 1.0cm below the surface of the water, is in the center of the third quadrant keeping a distance of 30cm from pool wall.

The rats were trained twice a day with an interval of 6 hours for consecutive 4 days. In each trial, the time that was required to escape onto the hidden platform was recorded as escape latency. On the next day of training (day 6), a probe trial was performed. In the probe trial, the time spent in the target quadrant, where the platform had been located during training, and the times rats traversed the quadrant were measured. Through the test, all the objects around the pool were kept invariable. The results were analyzed by a behavior software system.

2.6. Cell culture and experimental treatment

SH-SY5Y neuroblastoma cells were high passages from American Type Culture Collection (ATCC) and maintained in medium (DMEM/High Glucose, HyClone) containing 1% penicillin/streptomycin antibiotice and 10% fetal bovine serum (FBS), and were grown at 37℃ in the humidified atmosphere containing 5%CO_2. All experiments were carried out 24h at 37℃ after the cells were seeded onto plates. Taurine was freshly diluted with PBS. SH-SY5Y cells were pre-incubated with taurine for 24h before $A\beta_{1-40}$ was added. Assays for MTT, apoptosis and $\triangle\Psi m$ were performed at different time after $A\beta_{1-40}$ was added.

2.7. MTT assay

SH-SY5Y cells (1×105cells/ml) were treated with 20μM Aβ1-40 in the presence of taurine for 24h at 37°C, MTT solution 20μL was added to each well with the final concentration of 0.5mg/ml. After 3h at 37℃ incubation with MTT, cells were lysed in dimethyl sulfoxide (DMSO) and the amount of MTT to formazan was quantified by determining the absorbance at 570nm using a microplate reader.

2.8. Hoechst 33258 staining

After exposed to $A\beta_{1-40}$ for 24h, SH-SY5Y cells growing on 24-well plates were fixed with 4% paraformaldehyde for 30 min at room temperature, then washed with PBS 3 times and exposed to 1μg/ml Hoechst 33258 at room temperature in the dark for 15min. Samples were observed under a fluorescent microscope.

2.9. Measurement of $\triangle\Psi m$

$\triangle\Psi m$ was monitored using a cell permeable cationic dye, the fluorescent dye Rhodamine 123. Rhodamine 123 was added to each well to attain a final concentration of 5mM for 15 min at 37℃ after cells were treated with $A\beta_{1-40}$ for 24h. The cells were washed twice with PBS and collected. Cells were processed using by flow cytometry and data analysed by Cell Quest Pro Software.

FCM analysed the fluorescence intensity of the cells in each group, and the criterion of cell fluorescence intensity was 101. The strength higher than 101 is as the M2 region, on the contrary is M1 area. The M2 District means the proportion of normal cells, and M1 is the proportion of damaged cells. The intensity increase in the M1 area is considered as $\triangle\Psi m$ reduce.

2.10. Data analysis and statistics

Data are expressed as mean±SD. SPSS18 software was used for statistical analysis. Significance of difference was evaluated with One-Way ANOVA and LSD-t was used to statistically identify differences between groups. $P<0.05$ was considered statistically significant; n values indicate the number of samples. Each of the experiments in vitro was repeated at least three times.

3. Results

3.1. Effect of taurine on learning and memory abilities in AD rats induced by $A\beta_{1-40}$

Spatial learning was assessed by the time spent to find the hidden platform (escape latency). As shown in Table1, $A\beta_{1-40}$ -injected mice delayed the escape latency obviously compared to control group ($P<0.01$). The latencies of positive group (39.28 ± 1.26; $P<0.05$), taurine (3.2) group (39.08 ± 0.65; $P<0.05$) and taurine (4.79) group (39.11 ± 0.71; $P<0.05$) were obviously shorter than those of model group, which demonstrated that taurine was effective in attenuating spatial learning deficits in $A\beta_{1-40}$-treated rats.

Furthermore, the times of crossing where the platform had been located and the length of time in the target quadrant were both used as the norms to assess rats memory. Table 2 shows the results that model group displayed an obvious reduction in the time spent traversing where the platform had been (2.89 ± 0.56; $P<0.05$) and the swimming time in the target quadrant relative to the sham group (34.96 ± 1.43; $P<0.01$). However, the times of crossing platform and swimming time in the target quadrant of positive group (4.00 ± 0.33; 38.67 ± 1.12; $P<0.05$), Taurine (3.2) group (4.38 ± 0.37; 38.90 ± 1.18; $P<0.05$) and Taurine (4.79) group (4.10 ± 0.35; 38.92 ± 1.05; $P<0.05$) were markedly longer as compared to the model group (Table 1). These results demonstrated that taurine improved spatial memory against $A\beta_{1-40}$-induced toxicity.

Table1. Effect of taurine on learning and memory abilities in AD rats induced by $A\beta_{1-40}$ ($\bar{x}\pm s$)

Group(mmol/kg)	n	Letancy (s)	the times of traversing platform	swimming time in the third quadrant（s）
Control	1	38.07 ± 3.36	4.20 ± 1.03	39.31 ± 2.58
Sham	1	38.19 ± 3.26	4.10 ± 0.99	38.46 ± 3.66
Model	۹	$43.21\pm3.46^{**}$	$2.89\pm1.69^{*}$	$34.96\pm4.30^{**}$
Positive	1	$39.28\pm3.99^{\#}$	$4.00\pm1.05^{\#}$	$38.67\pm3.54^{\#}$
Taurine (1.6)	1	40.60 ± 3.80	2.80 ± 1.32	35.45 ± 3.37
Taurine (3.2)	۶	$39.08\pm5.23^{\#}$	$4.38\pm1.06^{\#}$	$38.90\pm3.35^{\#}$
Taurine (4.79)	1	$39.11\pm2.24^{\#}$	$4.10\pm1.10^{\#}$	$38.92\pm3.31^{\#}$

$^{*}P<0.05$，$^{**}P<0.01$ vs. control group; $^{\#}P<0.05$ vs. model group

3.2. Effect of taurine on SH-SY5Y cells against Aβ1-40-induced cytotoxicity

In order to better resemble the in vivo $A\beta_{1-40}$ -induced toxicity, we used a $A\beta_{1-40}$ -induced SH-SY5Y cell system. As estimated by MTT assays in SH-SY5Y cells, cell viability was markedly decreased in the presence of 20μmol $A\beta_{1-40}$ for 24h ($P<0.01$). However, when cells were pre-incubated with taurine (8, 20mmol) for 24h ($P<0.05$), cell toxicity was markedly attenuated in a dose-dependent manner (Table 2), which showed that taurine can protect SH-SY5Y cells against Aβ1-40-induced cytotoxicity.

Table 2. Effect of taurine against Aβ1-40-induced SH-SY5Y cell and Δ ψm (n=6, $\bar{x}\pm s$)

Group (mmol)	OD	cell viability (%)	Cell in M1 (%)
Control	0.52 ± 0.08	1.00 ± 0.12	20.37 ± 1.07
Model	$0.16\pm0.03^{**}$	$0.31\pm0.04^{**}$	$51.52\pm1.16^{**}$
Taurine (0.8)	0.25 ± 0.08	0.48 ± 0.09	37.26 ± 1.01
Taurine (8)	$0.45\pm0.14^{\#}$	$0.86\pm0.18^{\#}$	$21.46\pm1.39^{\#}$
Taurine (20)	$0.39\pm0.08^{\#}$	$0.77\pm0.97^{\#}$	$25.60\pm1.45^{\#}$

$^{*}P<0.01$ vs. control group; $^{\#}P<0.05$ vs.model group

3.3. Taurine protected SH-SY5Y cells against $A\beta_{1-40}$-induced apoptosis

Apoptotic cells were visualized by using the DNA-binding fluorescent dye Hoechst 33258. After treatment by Aβ1-40 at a concentration of 20μmol for 24h, cell nuclei became increasingly bright.

Finally, it decreased in size and fragmented into apoptotic bodies. In contrast, pre-incubated with taurine (8, 20mmol) for 24h, cells appeared significantly preserved and those alterations were remarkably attenuated (Figure 1).

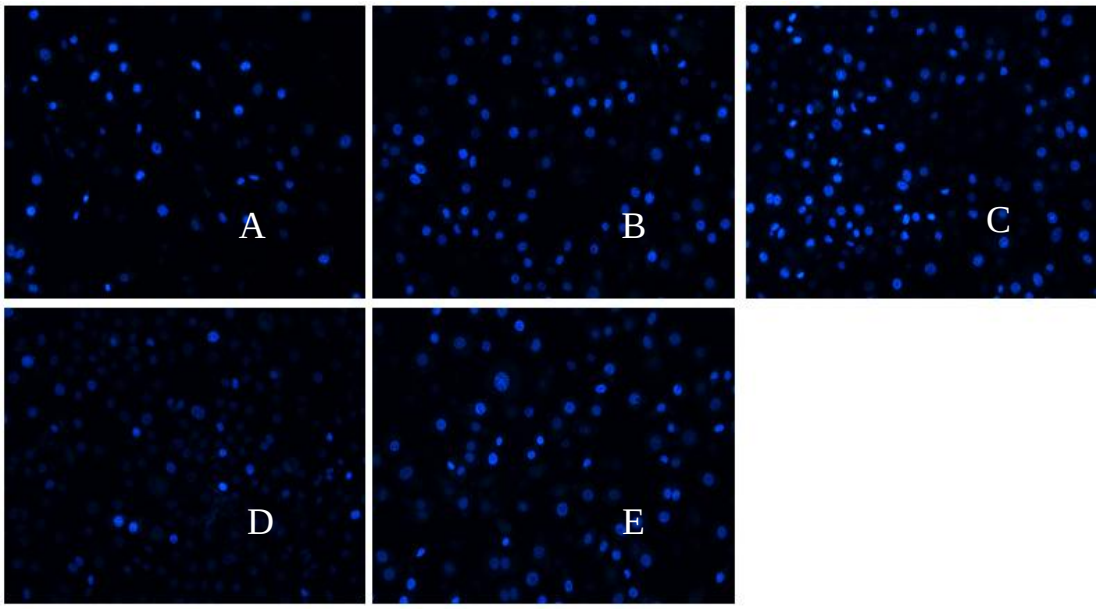

Figure 1. Morphological analysis of nuclear chromatin by Hoechst 33258. SH-SY5Y cells were treated with Aβ1-40 in the absence or in the presence of taurine ×40. (A) Control (B) After exposure to 20μmol Aβ1-40 for 24h, cell displayed condensed chromatin and apoptotic nuclei. (C-E) SH-SY5Y cells were pretreated with 0.8, 8, 20mmol taurine for 24h before exposure to 20μmol Aβ1-40 for 24h.

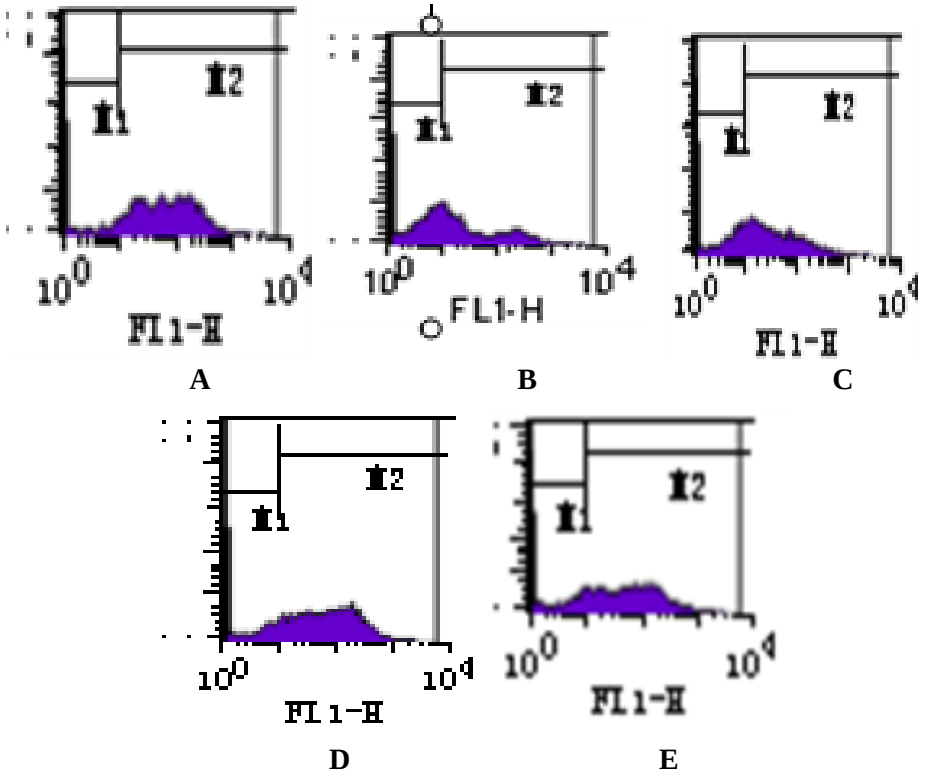

Figure 2. Effect of taurine on Aβ1-40-induced reduction of Δ ψm (original picture). (A) control (B) SH-SY5Y cells exposed 20μmol Aβ1-40 for 24h; (C-E) SH-SY5Y cells pretreated with 0.8, 8, 20mmol taurine for 24h followed by exposure to 20μmol Aβ1-40 for 24h.

3.4. Taurine prevented loss of ⊿ Ψm in SH-SY5Y cells

To assess the effect of taurine on the changes in $^{\triangle}\Psi m$ induced by $A\beta_{1-40}$, flow cytometric analyses were carried out using Rhodamine 123. After incubation of SH-SY5Y cells with 20μmol $A\beta_{1-40}$ for 24h, the $^{\triangle}\Psi m$ was detected by the weakening of the fluorescence intensity of Rhodamine 123. As compared to control cells, $A\beta_{1-40}$ treatment increased the Rhodamine 123 negative cells from 20.37%±1.07% to 51.52%±1.16% ($P<0.01$). Pretreatment with 8, 20mM of taurine protected cells against the $A\beta_{1-40}$-induced lowering of $^{\triangle}\Psi m$, decreasing Rhodamine 123 negative cells to 21.46%±1.39%; 25.6%±1.45% ($P<0.01$), respectively (Figure 2).

4. Discussion

Considerable evidence indicates that the Aβ is the collective access among all kinds of causes that induce AD and $A\beta_{1-40}$ is the critical factor of the formation and development of AD[10,11]. The Aβ aggregation located around neurons not only have a direct toxic effect on the neurons, but also enhance the susceptibility of neuronal cells to free radicals, nerve toxins and other harmful factors[12]. However, in the present studies we review the growing evidence for the role of soluble intracellular Aβ in the disease progression, with particular reference to Aβ found within the mitochondria. Once inside the nerve cells, Aβ is able to interact with a number of targets, including the mitochondrial proteins alcohol dehydrogenase and cyclophilin D, which is a component of the mitochondrial permeability transition pore (MPTP). Interference with the normal functions of these proteins results in mitochondrial collapse and ultimately cell death. Therefore, numerous publications have used the $A\beta_{1-40}$ aggregate injection rat model and induced cell model to study AD[13-15].

Taurine is a sulfur containing amino acid, also known as bovine cholic acid and bovine bilirubin. It was first isolated from bovine bile in 1827. Later, taurine was found to be a free amino acid in the central nervous system, and it is the main inhibitory transmitter in the nervous system[16]. Taurine was reported to be involved in many important physiological functions in the central nervous system, and could significantly promote nerve cell proliferation and differentiation[4]. Studies have found that, in the absence of oxygen, ischemia, and stress conditions, rodent brain taurine release increased significantly[17]. Intracerebroventricular infusion of $A\beta_{1-40}$ was demonstrated to induce spatial memory and learning impairment in AD animal models. It was reported that a single intracerebroventricular injection of $A\beta_{1-40}$ in mice induced markly amnesia as compared to the sterile saline-injected sham group. This confirmed that cognitive impairment was induced by $A\beta_{1-40}$ peptide itself, and was not attributable to an intracerebroventricular injection[18]. Our results showed that taurine, taken by oral lavage of 3.2mmol/kg and 4.79mmol/kg, improved spatial learning effectively across the 4-day acquisition training period. The $A\beta_{1-40}$-administered rats after taurine treatment showed a much better learning capability in finding the hidden platform by decrease of escape latency. In the memory probe trial, the $A\beta_{1-40}$-treated rats receiving taurine treatment performed better in searching for the target quadrant and the site where the platform was located as compared to the $A\beta_{1-40}$-administered rats. As a result of the current study on in vivo cognitive function, taurine has been shown to be potentially beneficial for the treatment of AD.

In our research, treatment of SH-SY5Y cells with Aβ1-40 caused a marked decrease in cell survival. Taurine remarkably reduced the cell death. In addition, exposed to 20μmol Aβ1-40 for 24h, SH-SY5Y cells showed typical characteristics of apoptosis, such as chromosome condensation. Taurine could reduce the apoptotic morphological changes in Aβ-induced injury in SH-SY5Y cells.

Previous studies have shown that apoptosis plays a pivotal role in AD pathogenesis[19]. And apoptosis in AD may be caused by accumulation of Aβ[20]. The main Aβ-induced apoptosis signaling pathways is Mitochondria. For example, patients with AD regularly exhibit declining mitochondrial energy metabolism, which may subsequently cause neuronal damage[21]. The increase of Aβ may impair mitochondrial function, leading to a significant decrease in $^{\triangle}\psi m$. Loss of $^{\triangle}\psi m$ causes the release of apoptosis-inducing factors which activate caspase cascade, cause brain energy production reduction, and enhance production of reactive oxygen species, and thereby contributing to apoptosis[22,23]. Further, activation many stressors such as oxidative stress, also causes neuronal apoptosis via the mitochondria-dependent pathway[24]. According to our study, a loss of $^{\triangle}\psi m$ was apparent in SH-SY5Y cells in the presence $A\beta_{1-40}$. And we also observed that taurine alleviated mitochondrial dysfunction through improving mitochondrial membrane potential.

5. Conclusion

With the experimental design and methodology used in the study, we investigated the therapeutic value of taurine in cognitive function and neuroprotective against Aβ-induced toxicity. Furthermore, we explored the therapeutic targets for the efficacy of taurine, and showed the underling mechanisms of taurine through regulating

mitochondrion-mediated apoptosis. In summary, taurine appears to be a prospective candidate for the prevention and treatment of Alzheimer's disease.

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