Taurine can Decrease Phosphorylated Tau Protein Levels in Alzheimer's Model Rats' Brains

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ABSTRACT

Background

Microtubule formation is a dynamic process and Tau proteins promote the assembly of tubulin monomers into microtubules. Hyperphosphorylation of some amino acids in tau proteins causes neuron starvation and finally cell death. Taurine is found in the brain and has neuroprotective effects.

Objective

Since the protective and therapeutic effects of Taurine on phosphorylated tau proteins level in the cerebellum and prefrontal cortex of rats induced by scopolamine have not been studied, we examined these effects.

Method

Adult male Wistar rats were randomly distributed into nine groups. For two weeks, Taurine-treated rats received different doses of Taurine (25, 50, and 100 mg/kg/day) before or after scopolamine injection. The phosphorylated tau protein level in the cerebellum and prefrontal cortex was determined by the enzyme-linked immunosorbent assay (ELISA) technique.

Result

Pretreatment with three doses of Taurine significantly decreased the phosphorylated tau protein level that increased by scopolamine in the prefrontal cortex ($p < 0.001$), as well as the cerebellum ($p < 0.001$). Moreover, high-dose administration of Taurine (100 mg/kg/day) after scopolamine injection significantly decreased phosphorylated tau protein level in the cerebellum ($p < 0.01$), as well as the prefrontal cortex ($p < 0.05$). However, there was not any significant change in the level of phosphorylated tau protein after Taurine treatment (25 and 50 mg/kg/day) in the cerebellum and prefrontal cortex.

Conclusion

It can be concluded that Taurine could attenuate the increase in phosphorylated tau protein induced by scopolamine in the brain of rats and usage of Taurine as a pretreatment complement could be more useful in the protection of neurons.

KEY WORDS

Cerebellum, ELISA, Prefrontal cortex, Scopolamine, Tau protein, Taurine

INTRODUCTION

Alzheimer's disease is associated with the hyperphosphorylated tau proteins.[1,2] Tau proteins promote the assembly of tubulin monomers into microtubules.[3] Disruption of this process may cause synaptic transmission dysfunction and neuronal death.[4] Dementia is associated with NFTs in the neocortex regions.[5] Therefore, produces of abnormal tau hyperphosphorylated proteins and the contributing mechanisms should be determined for the introduction of tau protein-based drugs.[6]

Administration of scopolamine [a nonselective muscarinic receptor antagonist in rodents leads to cholinergic dysfunction, impaired learning, and memory abilities, increased oxidative stress and synaptic irregularity, increased amyloid-β deposition and progress of tau phosphorylation, which are neuropathological symptoms of AD.[7,11-13] Several studies have shown that scopolamine induces overproduction of tau proteins in the brain of rats.[7,14-16]

Taurine, a 2-aminoethanesulfonic acid, is found at high concentrations in the brain of mammals and has multiple physiological functions.[2,17] Recently, it might be a candidate for the prevention and inhibition of neurodegenerative disorders.[18] Taurine has been reported to trigger the aggregation of tau fragments consisting of basic regions for microtubule binding.[19]

Also, anatomical data propose the disynaptic fronto-cerebellar connectivity in the rat and primary data in mouse suggest that between the prefrontal cortex and cerebellar nuclei there is a neural connection.[20,21] These studies suggest a cerebello-prefrontal network in rodents that revealing of that described in primates.[22]

METHODS

An experimental study was initiated after approval of Ethical Review Committee of Golestan University of Medical Sciences. The study was conducted from February 2018 to June 2019 on 72 male adult Wistar rats (weight, 180-220 g) were supplied by Pasteur Institute (Tehran, Iran). They were kept in a room with a 12:12 h dark/light cycle at an appropriate temperature (22±2°C) with free access to water and food.

Experimental protocol

Animals randomly were divided into 9 groups (n=8 per group):

1) Control: receiving no drugs

2) Saline+scopolamine: receiving saline (1 ml/kg/day, intraperitoneally (i.p.)) over 2 weeks, followed by scopolamine 3 mg/kg (9) administered once (i.p.) on the 15th day

3) Taurine+scopolamine (pretreatment group): receiving 25 mg/kg of Taurine per day (i.p.) for 2 weeks, followed by scopolamine injection on the 15th day.[23,24]

4) Taurine+scopolamine (pretreatment group): receiving 50 mg/kg of Taurine per day (i.p.) for 2 weeks, followed by scopolamine injection on the 15th day

5) Taurine+scopolamine (pretreatment group): receiving 100 mg/kg of Taurine per day (i.p.) for 2 weeks, followed by scopolamine injection on the 15th day

6) Scopolamine+saline: Scopolamine injection on the first day, followed by the injection of saline for 2 weeks from the 2nd day

7) Scopolamine+taurine (treatment group): scopolamine injection on the first day, followed by Taurine injection at 25 mg/kg dose from the 2nd day for 2 weeks.

8) Scopolamine+taurine (treatment group): scopolamine injection on the first day, followed by Taurine injection at 50 mg/kg dose from the 2nd day for 2 weeks.

9) Scopolamine+taurine (treatment group): scopolamine injection on the first day, followed by Taurine injection at 100 mg/kg dose from the 2nd day for 2 weeks.

Scopolamine hydrobromide (Tocris, UK) and Taurine (Sigma, Japan) were dissolved in sterile saline (0.9% NaCl) before use.

Collection of Brain Samples

Forty-eight hours after the end of experimental sessions, deep anesthesia with chloroform was induced to decapitate the rats; their brains were excised rapidly. The cerebellum and prefrontal cortex samples from 1 hemisphere were separated and homogenized in PBS solution and for 20 minutes were centrifuged at 5000 rpm. Afterward, the supernatants were used for phosphorylated tau protein assay by enzyme-linked immunosorbent assay (ELISA) technique.

Phosphorylated tau protein in the cerebellum and prefrontal cortex

ELISA assay was applied for the quantitative analysis of phosphorylated tau in the cerebellum and prefrontal cortex, based on the manufacturer's instructions (ZellBio Gmbh, Germany). The method of this kit was based on the biotin double-antibody sandwich. Color changes were measured at a wavelength of 450 nm with an ELISA reader. After comparing with the standard the levels of phosphorylated tau protein were determined and reported as pg/mL of tissue homogenized.

Data analyses were performed with SPSS 16.0 (Armonk, NY, USA). Normality tests of the data were performed using the Kolmogorov-Smirnov test. One-way ANOVA and post-hoc LSD tests were used for comparison of normal quantitative data between groups and data are expressed as mean±SD. The level of significance was set at 0.05.

RESULTS

Pretreatment effects of Taurine on phosphorylated tau protein

Phosphorylated tau protein significantly increased in the prefrontal cortex ($p < 0.001$), as well as the cerebellum ($p < 0.001$) after scopolamine administration alone, compared to normal control rats (fig. 1). Pretreatment with 3 doses of Taurine significantly attenuated the increased level of phosphorylated tau protein in the prefrontal cortex ($p < 0.001$) and cerebellum ($p < 0.001$), compared to the Sham saline+scopolamine group (fig. 1). Taurine pretreatment at a high dose (100 mg/kg/day) significantly reversed the increase in phosphorylated tau protein towards normal in the prefrontal cortex ($p< 0.001$), compared with the Sham saline+scopolamine group (fig. 1).

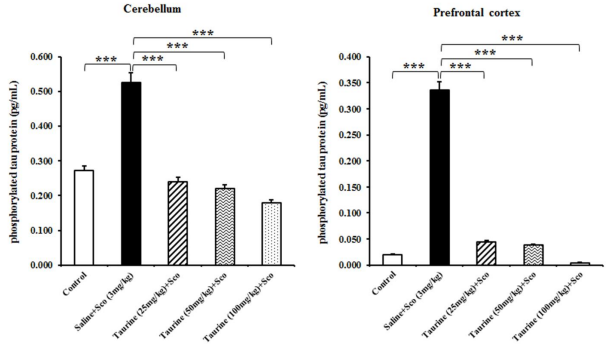

Figure 1. Pretreatment effect of taurine on phosphorylated tau protein (pg/mL) in the cerebellum and prefrontal cortex. Data are presented as mean±SD. ***P< 0.001 indicates a significant difference.

Treatment effect of Taurine on phosphorylated tau protein

A significant rise was observed in phosphorylated tau protein in the prefrontal cortex ($p < 0.05$), as well as the cerebellum ($p < 0.01$), compared to the controls. Taurine treatment (25 and 50 mg/kg/day) had no significant effects on phosphorylated tau protein in the prefrontal cortex and cerebellum in comparison with the scopolamine+saline group.

According to the LSD test, phosphorylated tau protein significantly reduced after high-dose treatment (100 mg/kg/day) in the cerebellum ($p < 0.01$) and prefrontal cortex ($p < 0.05$) in comparison with the scopolamine+saline group (fig. 2). However, high-dose Taurine (100 mg/kg/day) significantly mitigated the increased levels of phosphorylated tau protein ($p < 0.05$) in the prefrontal cortex in comparison to the scopolamine+saline group (fig. 2).

DISCUSSION

The findings of the current study revealed that scopolamine administration to rats increased phosphorylated tau proteins in the prefrontal cortex and cerebellum. The increase of phosphorylated tau was attenuated by

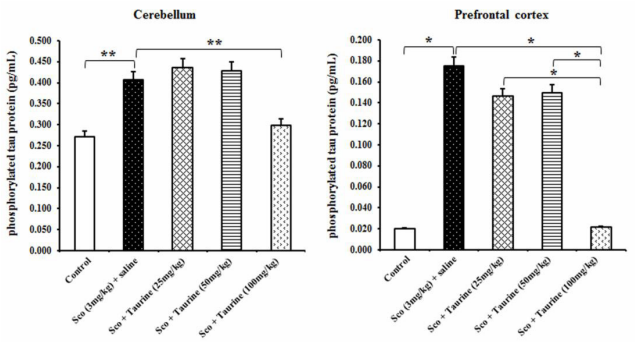

Figure 2. Treatment effects of taurine on the level of phosphorylated tau protein (pg/mL) in the cerebellum and prefrontal cortex. *P< 0.05 and **P< 0.01 represent significant differences.

pretreatment with Taurine. Furthermore, we showed that Taurine treatment decreased phosphorylated tau protein in the prefrontal cortex. However, in the cerebellum, treatment with Taurine at a high dose (100 mg/kg/day) decreased the phosphorylated tau protein levels.

Scopolamine is a nonselective muscarinic antagonist, capable of penetrating into the blood-brain barrier and producing an AD-like model, it is widely used to test potential drugs with anti-AD properties.[16,25,26] Furthermore, previous studies have shown that daily intraperitoneal injection of scopolamine (2 mg/kg for 4 weeks and/or 6 weeks) resulted in increased protein and mRNA levels of tau in the rat cortex and hippocampus.[7,16] It also significantly increased phosphorylated tau proteins in the rat hippocampus.[16,27] In another study, it is reported that scopolamine administration at 1 mg/kg for 2 weeks caused tau hyperphosphorylation in the mice hippocampus.[15] Consistent with these findings, scopolamine (3 mg/kg) increased the level of phosphorylated tau protein in the prefrontal cortex and cerebellum in our study.

Only a few reports have examined the effect of Taurine on the phosphorylated tau protein level. In a previous study, taurine (in vitro, 1 mM) induced the formation of tau polymers, whereas Taurine had no effects on phosphorylation of tau protein.[19] However, our study is the first to provide evidence that Taurine can decrease phosphorylated tau protein in the brain of scopolamine-treated rats.

Abnormal phosphorylation or hyperphosphorylation of tau proteins occurs in AD, which could be due to tau kinase upregulation or tau phosphatase downregulation.[3,28] Kinases, such as cAMP-dependent protein kinase, glycogen synthase kinase 3β (GSK3), and cyclin-dependent kinase 5, have major contributions to tau phosphorylation in the brain.[29] Under pathological and physiological conditions, GSK3 may help adjust the phosphorylation of these proteins.[30,31]

The mechanism of scopolamine effect on the increase of phosphorylated tau protein level maybe that upregulation of reactive oxygen species (ROS) by scopolamine-induced

GSK3 activation, which phosphorylates tau protein at multiple amino acid sites and finally forms NFTs, as a neuropathological symptom of AD.[13,15,32]

Since scopolamine activates GSK3 and causes tau phosphorylation, the impact of Taurine on tau protein modification via GSK3 was tested in vitro and in vivo. However, Santa-Maria et al. found no differences in the absence or presence of Taurine in terms of tau phosphorylation.[19] In addition, it has been recently reported that chronic treatment with Taurine (60 and 120 mg/kg, orally for 28 days) after intracerebroventricular streptozotocin injection did not change GSK3β expression in the cortex and hippocampus of rats.[17] Hence, GSK3β activation cannot be a mechanism responsible for the reduction of phosphorylated tau protein with Taurine in scopolamine-treated rats. Indeed, the mechanisms underlying the decreased levels of phosphorylated tau protein following Taurine treatment are not yet elucidated; however, Reeta et al. found that Taurine exerts neuroprotective effects through modulation of oxidative stress, inflammatory cytokines, cholinesterases and expression of rho kinase-II. Therefore, this study suggests the possibility of chronic Taurine administration in cognitive impairment of AD.[17]

Taken together, Taurine exhibited beneficial effects on phosphorylated tau protein in the brain. Meanwhile, the daily administration of Taurine for 14 days before scopolamine injection could prevent the rise in phosphorylated tau protein in the cerebellum and prefrontal cortex.

CONCLUSION

It can conclude that usage of Taurine as a pretreatment complement is more useful than the usage of it as a therapeutic drug. Also, phosphorylation of Tau protein that increases by scopolamine could attenuate by Taurine in the brain of rats.

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