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ORIGINAL PAPER

Anti-amyloidgenic and neurotrophic effects of tetrahydroxystilbene glucoside on a chronic mitochondrial dysfunction rat model induced by sodium azide

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Abstract
Alzheimer's disease (AD) is an irreversible neurodegenerative brain disorder with complex pathogenesis. Emerging evidence indicates that there is a tight relationship between mitochondrial dysfunction and β-amyloid (Aβ) formation. 2,3,5,4′-Tetrahydroxystilbene-2-O-β-D-glucoside (TSG) is one of the main active components extracted from *Polygonum multiflorum*. The purpose of the present study was to investigate the effects of TSG on Aβ production and neurotrophins in the brains of rats by using a mitochondrial dysfunction rat model induced by sodium azide (NaN_3), an inhibitor of mitochondrial cytochrome c oxidase (COX). NaN_3 was administered to rats by continuous subcutaneous infusion for 28 days via implanted osmotic minipumps to establish the animal model. TSG was intragastrically administered starting 24 h after the operation. The activity of mitochondrial COX was measured by a biochemical method. The content of Aβ 1-42 was detected by ELISA. The expression of neurotrophic factors was determined by Western blot and immunohistochemistry. The results showed that NaN_3 infusion for 28 days induced a decrease in mitochondrial COX activity, an increase in Aβ 1-42 content and the expression of amyloidogenic β-amyloid precursor protein (APP), beta-site APP cleaving enzyme 1 (BACE1) and presenilin 1 (PS1), and a decline in the expression of neurotrophins in the hippocampus of rats. Intragastrical administration of TSG elevated mitochondrial COX activity, decreased Aβ 1-42 content and the expression of APP, BACE1 and PS1, and enhanced the expression of nerve growth factor, brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B (TrkB) in the hippocampus of NaN_3-infused rats. These findings suggest that TSG may be beneficial in blocking or slowing the progression of AD by enhancing mitochondrial function, decreasing Aβ production and increasing neurotrophic factors at some extent.

Keywords Tetrahydroxystilbene glycoside · Alzheimer's disease · Mitochondrial dysfunction · β-amyloid · Neurotrophic factor · Sodium azide

Introduction

Alzheimer's disease (AD) is an irreversible neurodegenerative brain disorder [1]. The manifestation of AD is inevitably progressive and terminates in mental and functional incapacity and death, and the pathogenesis of AD is complex. Although the cause and pathogenesis of AD are not well understood, the mitochondria cascade hypothesis and amyloid hypothesis are prevalent in AD research. It has been reported that the decreased expression and activity of mitochondrial cytochrome c oxidase (COX, respiratory chain complex IV) are found in postmortem brain tissues from AD patients [2]. Additionally, emerging evidence indicates that there is a tight relationship between mitochondrial dysfunction and β-amyloid

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(Aβ) formation [3, 4]. Inhibition of mitochondrial complex IV induced by sodium azide (NaN_3) could affect amyloidogenic β-amyloid precursor protein (APP) metabolism. Conversely, Aβ exposure could accelerate mitochondrial damage [5, 6].

Neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are critical molecules that have been implicated in the pathogenesis of AD [7]. Both NGF and BDNF are affected early in the disease, and this is thought to initiate a cascade of events that exacerbates pathology and leads to the symptoms of dementia [8]. A reduction in BDNF levels or tropomyosin-related kinase B (TrkB)-mediated BDNF signaling has also been shown to reduce neuronal protection and increase APP [9].

2,3,5,4′-Tetrahydroxystilbene-2-O-β-d-glucoside (TSG) is the main component of *Polygonum multiflorum*, which has been widely used in the Orient as an anti-aging agent since ancient times. TSG is structurally identified (Fig. 1). Our previous studies found that TSG improved learning and memory abilities in APP transgenic mice and aged rats [10, 11], and TSG increased the number of synapses and elevated the expression of synaptophysin in the hippocampus of aged rats [10]. TSG has been developed as a new drug (Taisi capsule) to treat AD by our laboratory and is now under phase III clinical trials in China. However, it remains unclear whether TSG has an effect on mitochondrial dysfunction, and the mechanisms underlying TSG-mediated neuroprotection require further elucidation.

NaN_3 is a highly toxic substance and has been widely used as an inhibitor of mitochondrial COX [12, 13]. Chronic NaN_3-induced mitochondrial poisoning is suitable for producing AD-like symptoms in rats and testing neuroprotective drug candidates [14]. In the present study, we replicated a mitochondrial dysfunction rat model induced by chronic infusion of NaN_3 and investigated the effects of TSG on changes in mitochondrial COX activity, APP processing, and neurotrophic factors in the brain for the purpose of further understanding the effect of TSG on mitochondrial dysfunction-induced AD-like pathological changes.

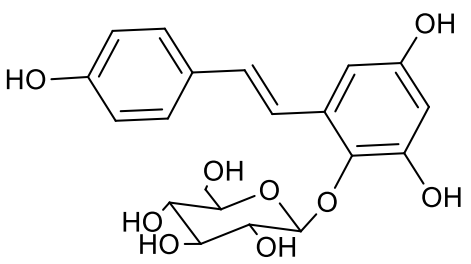

Fig. 1 Structure of 2,3,5,4′- tetrahydroxystilbene-2-O-β-D-glucoside (TSG)

Materials and methods

Drug and reagents

TSG, with a molecular weight of 406, was extracted from the root of *P. multiflorum* in our department, according to a previously described procedure [15]. NaN_3 was obtained from Ameresco Co., USA. Antibodies against APP and presenilin 1 (PS1) were purchased from Sigma-Aldrich, USA; NGF, BDNF and TrkB antibodies were from Abcam, UK; and beta-site APP cleaving enzyme 1 (BACE1) was from Santa Cruz, USA. All other reagents were from commercial suppliers and of standard biochemical quality.

Animals

A total of 56 adult male Sprague–Dawley rats weighing 390 ± 20 g were purchased from Vital River Laboratories, China. Rats were housed under a 12/12-h dark/light cycle and standard pathogen-free conditions. They had free access to food and water throughout the entire experiment. All experiments followed the requirements of the Provisions and General Recommendations of the Chinese Experimental Animal Administration Legislation. The suffering and the number of animals were minimized in all experimental conditions.

Animal model establishment and drug treatment

NaN_3 was dissolved in sterile normal saline and applied by continuous subcutaneous delivery via an ALZET® osmotic minipump (type: 2ML4; Alza Co., USA). The minipumps were kept in sterile 0.9% saline at 37 °C overnight before the operation. Rats were anesthetized by an intraperitoneal injection of 10% chloral hydrate. The minipumps containing NaN_3 were implanted under the dorsal skin of rats and kept for 28 days with a delivery rate of 2.5 μl/h.

The rats were randomly divided into 4 groups, with 14 in each group—(1) the control group, the minipumps were filled with sterile normal saline; (2) the model group, the minipumps supplied NaN_3 at 0.5 mg/kg/h; (3) the TSG-L group, NaN_3 0.5 mg/kg/h + TSG 60 mg/kg; and (4) the TSG-H group, NaN_3 0.5 mg/kg/h + TSG 120 mg/kg.

TSG powder was dissolved in distilled water and intragastrically administered to rats from 8:00–9:00 am daily for 27 days starting 24 h after the minipump implantation surgery. The control and model groups received an equal volume of distilled water every day. Three rats from each group were killed for histological analysis, and the rest of

the rats were killed for analysis of biochemical changes after NaN_3 delivery was complete.

Isolation of mitochondria

A protocol adapted from Racay et al. [16], with some modifications, was used to prepare metabolically active mitochondria from rat hippocampus. All procedures were carried out on ice. Dissected tissue was homogenized in ice-cold homogenization buffer (10 mM Tris, 320 mM sucrose, 1 mM EDTA·2 K^+, pH 7.4) using a disperser (IKA Works, Germany). Homogenates were centrifuged at 400×*g* for 5 min, and supernatants were collected. The resulting pellets were resuspended and centrifuged again at 400×*g* for 5 min. The combined supernatants were centrifuged at 12,000×*g* for 10 min. The mitochondria pellets were then lightly rinsed with 0.25 M sucrose and suspended in mitochondrial cryopreservation solution (Genmed Scientifics Inc., USA) to yield aliquots containing approximately 5 mg/ml protein and kept at −80 °C until use.

Measurement of mitochondrial COX activity

The activity of mitochondrial COX was determined spectrophotometrically using the Cytochrome c Oxidase Assay kit (Genmed Scientifics Inc., USA) following the manufacturer's instructions. Briefly, reactions were started by the addition of ferrocytochrome c. The oxidation of cytochrome c was monitored at 550 nm with a WFZ800-D3B UV/VIS spectrophotometer (Beijing Rayleigh Analytical Instrument Co., China). The reduction in absorbance was measured for 1 min. The relative COX activity was expressed as percentage difference versus the control group.

β-amyloid content detection

The cerebral cortex was added to a 4× mass of cold 50 mM Tris–Hcl (pH 8.0) and homogenized thoroughly with a disperser (IKA Works, Germany). Each sample was added with 0.5 ml 10 M guanidine-HCl. The homogenates were incubated at room temperature for 3.5 h, and then centrifuged at 14,000×*g* for 25 min at 4 °C. The supernatant was diluted to reduce the concentration of guanidine-HCl to 0.5 M. The content of Aβ 1-42 was determined using a sandwich Aβ 1-42 high-sensitivity test ELISA kit (Immuno-Biological Laboratories, Japan).

Western blot assay

Protein concentrations were determined with a bicinchoninic acid assay kit (Applygen Technologies Inc. China). Equal amounts of protein were loaded in each well of a 12% sodium dodecyl sulfate/polyacrylamide gel electrophoresis gel, electrophoresed with a Tris–glycine running buffer, transferred to a polyvinylidene difluoride membrane and immunoblotted with the antibodies against APP (dilution 1:500), BACE1 (1:500) and NGF (1:800). Membranes were then incubated with the appropriate secondary antibodies (1:2000). The immune complex was detected using ECL Western blotting detection reagents (Millipore Co., USA). The intensity of the bands on the membranes was analyzed using Image J software. β-actin was used to normalize against gel loading variability.

Immunohistochemistry

The rats were anesthetized with 10% chloral hydrate and perfused transcardially. The brains were cut coronally into sections (35 μm thick) with a cryotome (Shandon cryotome FE & FSE, Thermo Fisher Scientific, UK). Free-floating sections were blocked with 0.3% H_2O_2 for 20 min, and nonspecific sites were blocked with bovine serum albumin for 30 min at room temperature. Sections were then incubated overnight at 4 °C with the primary antibodies against PS1 (1:600), NGF (1:500), BDNF (1:400) and TrkB (1:500). After washing with PBS, sections were subsequently incubated with biotin-labeled secondary antibodies for 2 h at room temperature. The immunoreaction was detected using horseradish peroxidase-labeled antibodies for 1 h at 37 °C and visualized with the diaminobenzidine tetrachloride system. The images were observed using a microscope (Olympus BX60, Japan), and brown-colored cells were identified as positive. For quantification, all slides were evaluated by a single investigator who was blinded to the treatment regimen. Three rats were taken from each group, 3 slides from each rat, and a total of 9 sections from each group were read under a microscope. The number, density and area of the positively stained cells were measured with Image-pro Plus 6.0 software (Media Cybernetics, Inc. USA).

Statistical analysis

SPSS 16.0 software was used for the statistical analyses. All data were expressed as the mean ± standard error of mean (SEM). The significance of difference of mean between more than two groups was determined using one-way ANOVA followed by Tukey's post hoc test. A P value of < 0.05 was considered statistically significant.

Results

TSG increases mitochondrial COX activity in the hippocampus of NaN_3-infused rats

In the present study, NaN_3 infusion for 28 days resulted in a decline in mitochondrial COX activity in the hippocampus of rats compared with the control group. Intragastric administration of TSG for 27 days starting 24 h after the minipump implantation surgery increased the activity of mitochondrial COX in the hippocampus of NaN_3-infused rats, and the 60 mg/kg group showed a statistically significant difference ($P < 0.05$; Fig. 2).

TSG decreases Aβ 1-42 burden in the cerebral cortex of NaN_3-infused rats

The content of Aβ 1-42 in the cerebral cortex of rats was measured by an ELISA method. The results demonstrated that NaN_3 infusion significantly increased Aβ 1-42 content in the cerebral cortex compared with the control rats ($P < 0.01$). Administration of TSG decreased Aβ 1-42 content in the NaN_3-infused rats ($P < 0.05$, $P < 0.01$; Fig. 3).

TSG reduces APP expression in the hippocampus of NaN_3-infused rats

Western blot analysis was used to detect the expression of APP. The results revealed that the APP protein level was significantly elevated in the hippocampus of NaN_3 model rats compared with the control group ($P < 0.01$). The TSG treatment (60 and 120 mg/kg) evidently resulted in declined expression of APP in the hippocampus of NaN_3-infused rats ($P < 0.05$; Fig. 4).

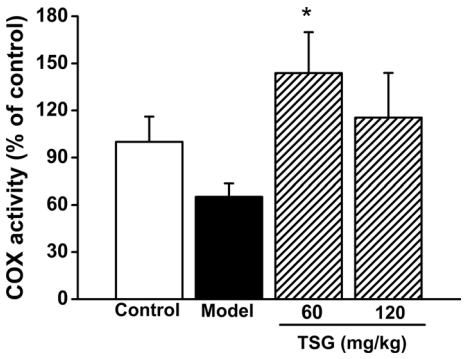

Fig. 2 Effects of TSG on the activity of mitochondrial cytochrome c oxidase (COX) in the hippocampus of NaN_3-infused rats. TSG was intragastrically administered to the rats after ALZET® osmotic minipumps containing NaN_3 were implanted under the dorsal skin. The mitochondria were isolated from the hippocampus of rats after 28 days of continuous and constant-speed NaN_3 infusion via minipumps. Mitochondrial COX activity was determined by spectrophotometry. The relative COX activity is expressed as a percentage change versus the control group. Data represent the mean ± SEM of 6 rats from each group. *$P < 0.05$, TSG group versus the model group

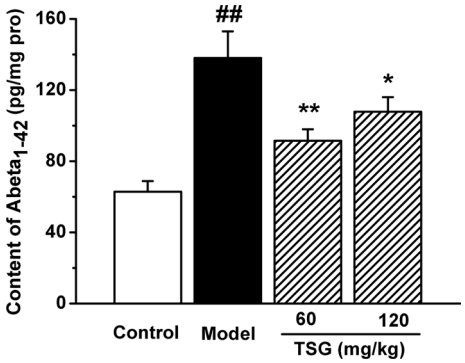

Fig. 3 Effects of TSG on Aβ 1-42 content in the cerebral cortex of NaN_3-infused rats. The content of Aβ 1-42 in the cerebral cortex of rats was determined by ELISA. Data represent the mean ± SEM of 6 rats from each group. ##$P < 0.01$, NaN_3 model group versus the control group; *$P < 0.05$, **$P < 0.01$, TSG group versus the model group

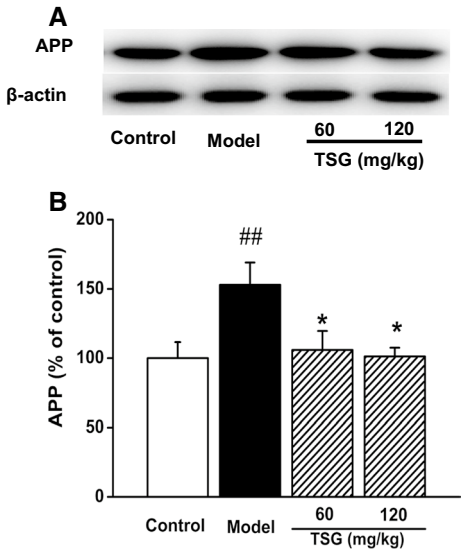

Fig. 4 Effects of TSG on APP expression in the hippocampus of NaN_3-infused rats. Western blotting was used to detect the expression of APP. **a** Representative images of immunoblots for APP. **b** Quantitative analysis of APP expression. Raw data were converted to relative values, and β-actin was used as the internal reference. The control group was taken as 100%, and others were expressed as a percentage of the control group. Data represent the mean ± SEM of 3 rats per group. ##$P < 0.01$, NaN_3 model group versus the control group, *$P < 0.05$, TSG group versus the model group

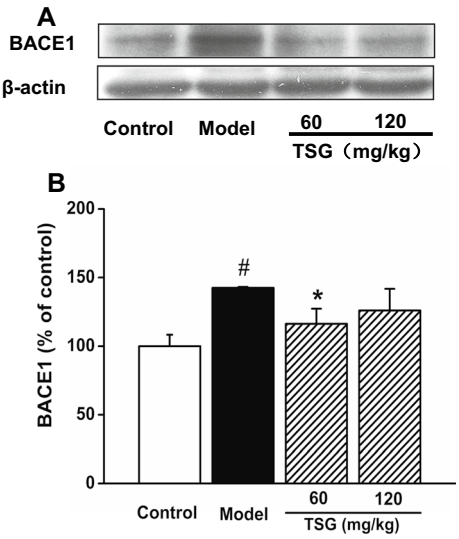

Fig. 5 Effects of TSG on BACE1 expression in the hippocampus of NaN_3-infused rats. Western blotting was used to detect the expression of BACE1 in the hippocampus. **a** Representative images of immunoblots for BACE1. **b** Quantitative analysis of BACE1 expression. β-actin was used as the internal reference; the control group was taken as 100%, and others are expressed as a percentage of the control group. Data are presented as the mean ± SEM of 3 rats per group. $^{\#}P < 0.05$, NaN_3 model group versus the control group; $^{*}P < 0.05$, TSG group versus the model group

TSG decreases BACE1 and PS1 expression in the hippocampus of NaN_3-infused rats

The results from Western blotting showed that the expression of BACE1 (β-secretase) was significantly higher in the hippocampus of model rats after NaN_3 infusion compared with the control group ($P < 0.05$). TSG administration decreased BACE1 expression in the hippocampus of NaN_3-infused rats, and the 60 mg/kg group showed a statistically significant difference ($P < 0.05$; Fig. 5).

An immunohistochemical method was used to assess the expression of PS1 (the catalytic component of γ-secretase). The results showed that NaN_3 infusion significantly increased the number of PS1-positive cells in the hippocampal CA1 region ($P < 0.05$), whereas administration of TSG decreased the number and density of PS1 positive cells in the hippocampal CA1 region compared with NaN_3 model rats ($P < 0.05$; Fig. 6).

TSG enhances the expression of neurotrophins in the hippocampus of NaN_3-infused rats

The results from Western blotting indicated that the hippocampal NGF level was decreased in the NaN_3-infused rats compared with the control group. Administration of TSG obviously increased the hippocampal NGF expression in the NaN_3-infused rats ($P < 0.05$; Fig. 7a, b). The immunohistochemical analysis of NGF expression in the hippocampus also exhibited a similar trend as the Western blots (Fig. 7c).

Immunohistochemical staining showed that the number, density and area of BDNF-positive cells in the hippocampal CA1 region were lower after chronic NaN_3 infusion. TSG treatment remarkably elevated the expression of BDNF in the CA1 region of NaN_3-infused rats ($P < 0.05$; Fig. 8).

We also probed the expression of TrkB, a high-affinity receptor of BDNF, using the immunohistochemical method. The results showed that the number, density and area of TrkB-positive cells declined in the hippocampal CA1 region after NaN_3 infusion; the treatment of TSG increased the number, density and area of TrkB positive cells in this region of NaN_3-infused rats ($P < 0.05$; Fig. 9).

Discussion

The implication of mitochondria in neurodegeneration is widely accepted [17]. Mitochondrial dysfunction is one of the earliest and most prominent features in vulnerable neurons in the brain of AD patients [18]. The most consistent defect of mitochondrial electron transport chain enzymes in AD is the deficiency in COX activity [19]. One consequence of COX inhibition is a reduced yield of adenosine triphosphate (ATP) [20]. Tissue-specific decreases in ATP could contribute to the preferential destruction of nerve and skeletal muscle cells via necrosis and/or apoptosis [21, 22]. Furthermore, COX inhibition increases reactive oxygen species (ROS) [23]. NaN_3 is a specific inhibitor of COX, which is also a rate-limiting enzyme in oxidative phosphorylation [20]. In the present study, we established a mitochondrial dysfunction rat model by NaN_3 infusion. NaN_3 is a highly toxic substance and will induce high mortality rates if it is injected at high dosage in a short time. Therefore, we chose osmotic minipumps containing NaN_3 and implanted them under the dorsal skin of rats for 28 days. Using this method, NaN_3 could be infused into the rat body in a continuous, constant-speed, low-dose and long-term manner. We found that chronic NaN_3 infusion reduced the activity of mitochondrial COX in the hippocampus of rats. This is consistent with previous results of other investigators [19]. Intragastric administration of TSG for 27 days significantly increased mitochondrial COX activity in NaN_3-infused rats. Luques et al. reported that chronic NaN_3 administration decreased mitochondrial COX activity in the brain and skeletal muscles via direct inhibition of the enzyme's catalytic activity [24]. We propose that TSG may directly act on COX and protect mitochondrial function.

In the present study, we found that Aβ 1-42 content was significantly increased in the cerebral cortex of NaN_3-infused rats. It is known that Aβ 1-42 is the main component of

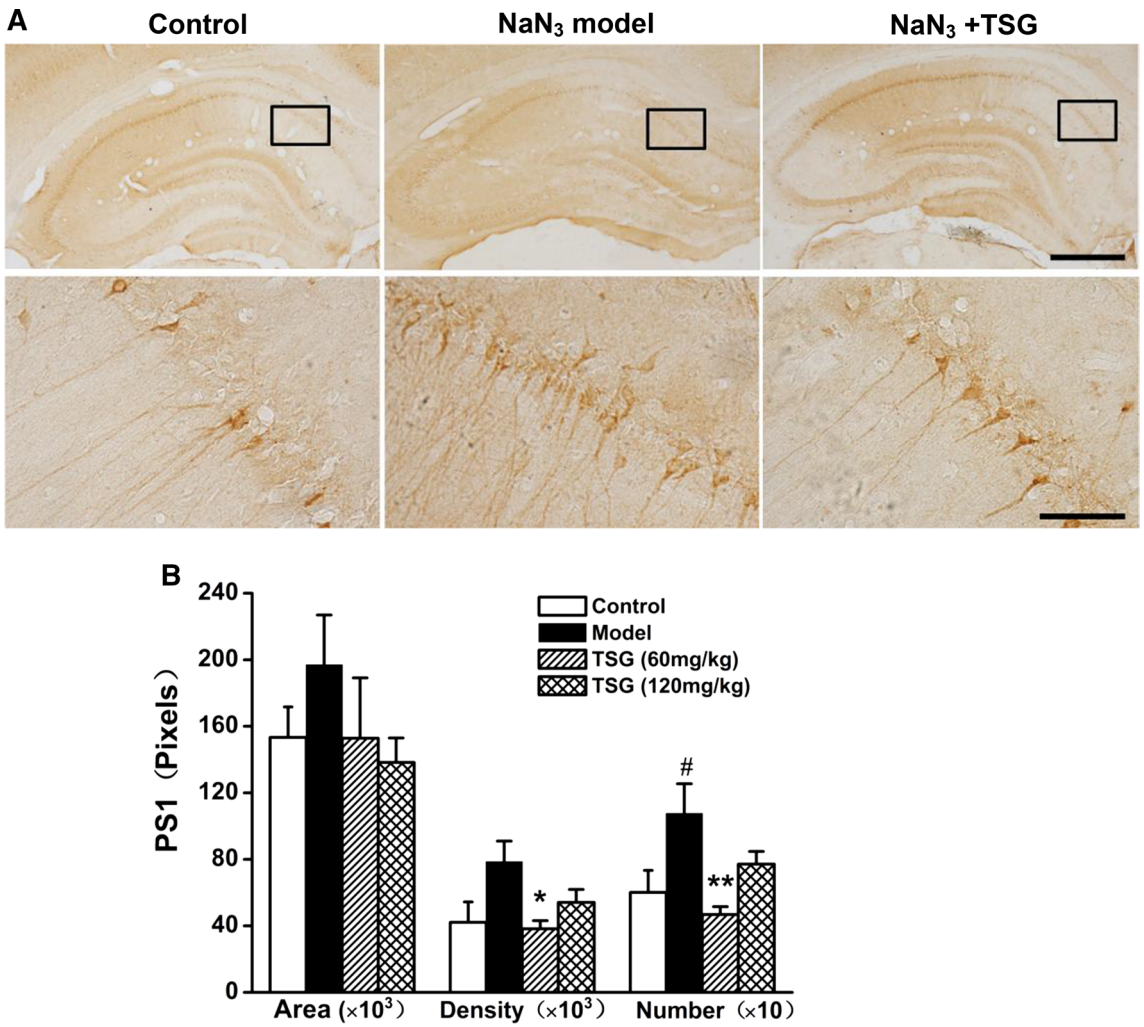

Fig. 6 Effects of TSG on PS1 expression in the hippocampal CA1 area of NaN_3-infused rats. PS1-positive neurons in the hippocampus of rats were stained and appear as brown particles by the immunohistochemical method. **a** Representative images of immunohistochemistry for PS1. Scale bar in the upper row = 500 μm, in the lower row = 50 μm. **b** Image pixel analysis of the number, density and area of PS1 positive neurons in hippocampal CA1 area. Data are expressed as the mean ± SEM from 9 sections per group (3 sections per rat, 3 rats each group). $^{\#}P < 0.05$, NaN_3 model group versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, TSG groups versus the model group

senile plaques, which are one of the characteristic pathological features of the AD brain. Leuner et al. reported that mitochondria dysfunction-derived increases in ROS lead to enhancement of Aβ 1-42 formation [3]; Aβ overproduction, in turn, causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins [25], thus forming a vicious circle. In the present study, we found that TSG significantly decreased the content of Aβ 1-42 in the cerebral cortex of NaN_3-infused rats, suggesting that TSG may be beneficial for AD therapy.

APP is a highly conserved integral membrane protein and is constitutively cleaved by α-, β-, and γ-secretases during its maturation and processing [26]. Aβ is the cleavage product of APP. To explore the mechanism by which TSG lowers Aβ, we measured the APP content in the brains of NaN_3-infused rats. It has been reported by other investigators that NaN_3 induces the production of the amyloidogenic C-terminal fragment of APP in COS-1 cells [27] and increases intracellular APP holoprotein in vitro [13]. On the other hand, APP can also affect mitochondria. APP is reported to localize to mitochondrial membranes, block the transport of nuclear-encoded mitochondrial proteins to mitochondria, interact with mitochondrial proteins, disrupt electron transport chain activities, increase ROS production, and thus cause mitochondrial dysfunction [28]. In the present study, we found that APP expression in the hippocampus of rats was significantly increased after NaN_3 infusion, but were lower when combined with TSG treatment. Because APP is the source of Aβ, the inhibitory effect of TSG on APP expression may decrease the source

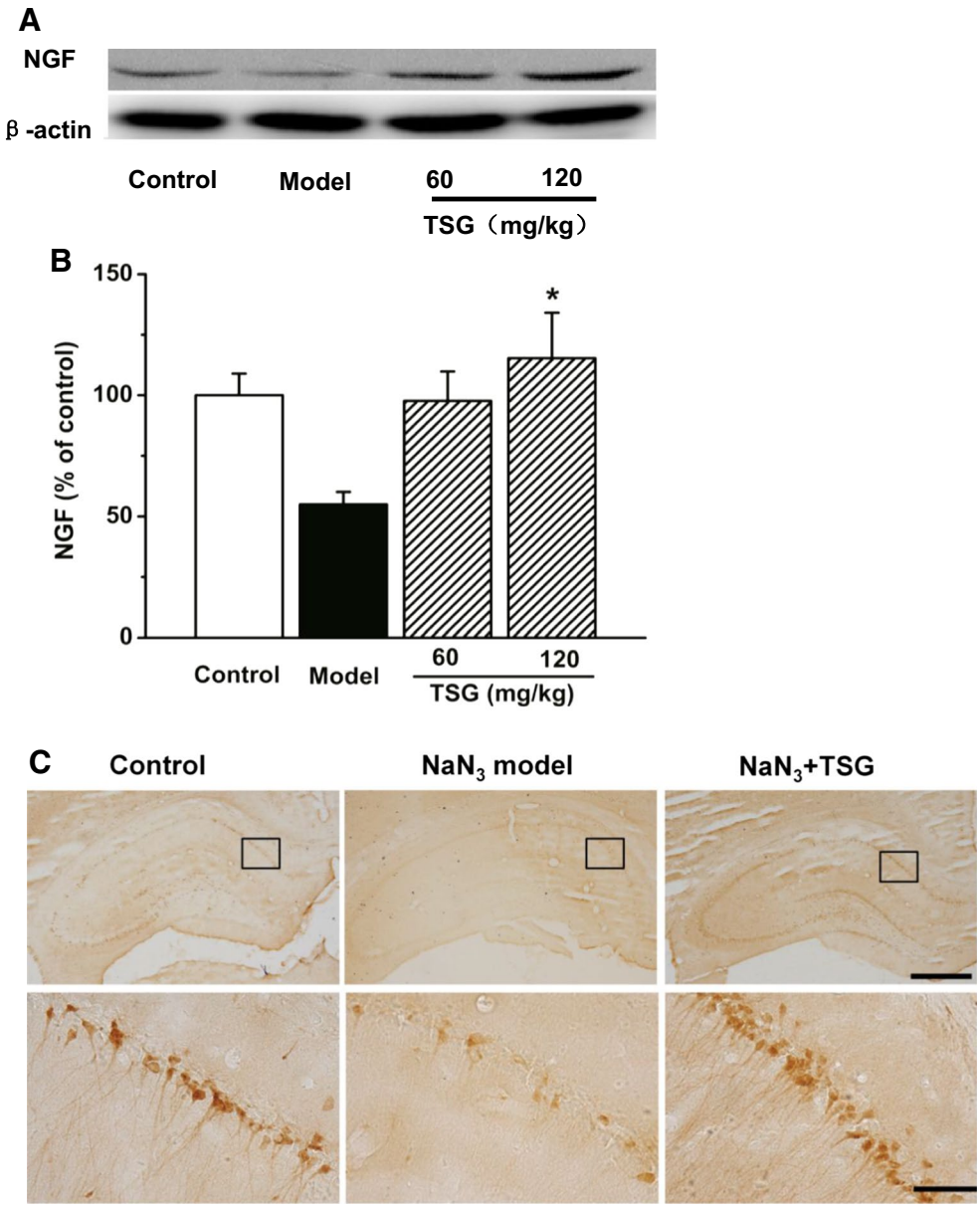

Fig. 7 Effects of TSG on NGF expression in the hippocampus of NaN_3-infused rats. **a** Representative images of Western blots for NGF in the hippocampal tissue. **b** Quantitative analysis of NGF expression from Western blot images. β-actin was used as the internal reference; the control group was taken as 100%, and the others are expressed as a percentage of the control group. Data represent the mean ± SEM of 3 rats per group. $*P < 0.05$, TSG group versus the model group. **c** Representative images of immunohistochemistry for NGF in the hippocampus. Scale bar in the upper row = 500 μm, in the lower row = 50 μm.

of Aβ production. This may be one of the mechanisms by which TSG reduces Aβ content.

In addition to APP expression, we also investigated the effects of TSG on APP processing in NaN_3-infused rats. Aβ is cleaved from APP by β- and γ-secretase [29]. BACE1 (β-secretase), a key rate-limiting enzyme for regulating Aβ production, cleaves APP at the N-terminal end, producing a 99 amino acid APP C-terminal fragment, which is further cleaved within the transmembrane domain by γ-secretase, resulting in Aβ production [30]. It has been reported that the levels of BACE1 expression and activity are elevated in sporadic AD brains [31, 32]. Mitochondrial respiratory inhibition and oxidative stress elevate BACE1 activity in vivo in the rat retina [33]. Mitochondrial dysfunction is the basic mechanism underlying the induction of oxidative stress [30], and oxidative stress up-regulates PS1 (a catalytic subunit of γ-secretase) in lipid rafts in neuronal cells [34] and increases the expression and activity of BACE in NT2 neurons [35]. In the present study, we found that the expression of BACE1 and PS1 was increased in the hippocampus

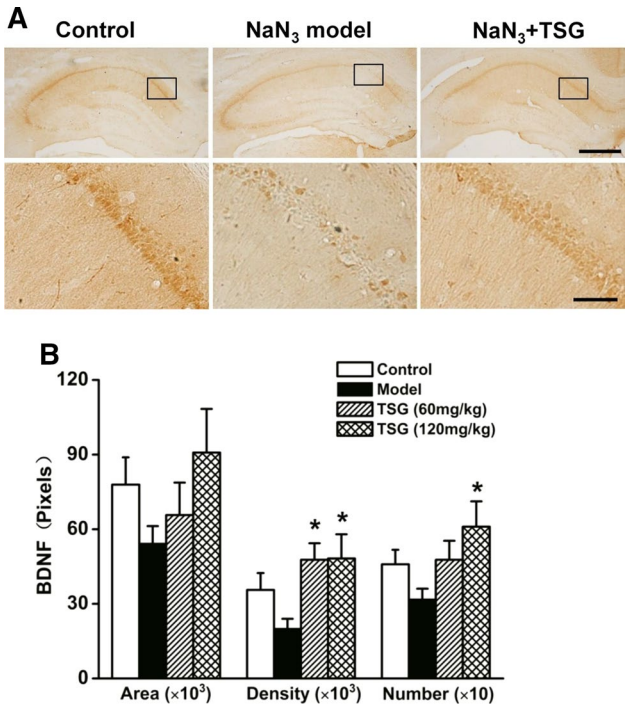

Fig. 8 Effects of TSG on BDNF expression in the hippocampal CA1 region of NaN_3-infused rats. **a** Representative images of immunohistochemistry for BDNF in the hippocampus. Scale bar in the upper row = 500 μm, in the lower row = 50 μm. **b** Image pixel analysis of the number, density and area of BDNF-positive neurons in the hippocampal CA1 area. Data are expressed as the mean ± SEM from 9 sections of each group (3 sections per rat, 3 rats each group). *$P < 0.05$, TSG groups versus the model group

of NaN_3-induced model rats, and these results are consistent with the reports of other investigators [4, 33]. Administration of TSG decreased BACE1 and PS1 expression in the hippocampus of NaN_3-infused rats. This may be another mechanism by which TSG reduces Aβ content.

Neurotrophins are critical molecules that support the development, differentiation, maintenance and plasticity of brain function throughout life [8]. BDNF binds to its receptor TrkB and makes a major contribution to cognition, learning and memory through neurotrophic support and modulation of synaptic plasticity [36]. NGF is an important substance in nutrition for the development, survival and maintenance of neurons in cholinergic basal forebrain [35]. Both NGF and BDNF are decreased early in AD, and this is thought to initiate a cascade of events that exacerbates pathology and leads to the symptoms of dementia [8]. Lee et al. observed BDNF decrease in both AD and mild cognitive impairment patients [37]. NGF deficiency in the brain accelerates Aβ deposits and Aβ-induced toxicity and induces apoptosis, death and dysfunction of neurons [38], whereas exogenous administration of BDNF counteracts the neurotoxic effects of Aβ in vitro and in vivo [39]. Moreover, there are some reports of a relationship between mitochondria and neurotrophins. Kim et al. found that rotenone, an inhibitor of mitochondrial complex I, decreases the level of intra- and extracellular BDNF in SH-SY5Y cells, suggesting that complex I dysfunction may disrupt BDNF. Because their previous studies showed that oxidative stress decreased BDNF levels, they hypothesize that oxidative stress induced by complex I dysfunction may underlie its inhibitory effect on BDNF expression [40]. BDNF, in turn, may also affect mitochondria. Markham et al. reported that BDNF increases the respiratory efficiency of brain mitochondria [41]. Burkhalter et al. have shown that BDNF, through an intricate pathway, induces mitochondrial biogenesis and favors ATP homeostasis in neurons [42]. In the present study, we found that NaN_3 infusion led to a decrease in the expression of NGF, BDNF and its receptor TrkB in the hippocampus of rats, indicating that mitochondria dysfunction induced neurotrophin deficiency. TSG treatment increased the expression of NGF, BDNF and TrkB in the hippocampus of NaN_3-infused rats, suggesting that TSG may be beneficial for neuron protection and AD therapy.

In addition, this study also found that the increase of PS1 and the decrease of BDNF and TrkB were greatest in the CA1 region of the hippocampus of NaN_3-induced model rats. It is known that the hippocampal formation consists of several cytoarchitectonically distinct subdivisions, including the hippocampus proper (which is subdivided into fields CAl, CA2, and CA3), the dentate gyrus, and the subicular complex. The CA1 subfield of the hippocampus has received the most attention in AD research. The CA1 region of the human hippocampus has undergone a greater enlargement in area and contains almost twice as many pyramidal cells as other fields of the hippocampal formation [43, 44]. Padurariu et al. quantified neuronal density in the four specific areas of the hippocampus (CA1–CA4) of AD brains. They found a significant reduction of neuronal density, especially in the CA1 area, compared to an age-matched control group [45]. Additionally, the characteristic degenerative processes of AD do not equally affect all cell types. The pyramidal cells in the CA1 region of the hippocampus seem to be more vulnerable to neurofibrillary tangle formation and neurodegeneration than cells of other hippocampal areas [46]. A lesion confined to CA1 of the hippocampus essentially breaks the chain of information processing that begins at the dentate gyrus and ends in the subicular complex and entorhinal cortex. This lesion, while spatially limited, would be expected to have a profound influence on the function of the hippocampal formation [47]. In our present study, the immunohistochemical results showed that the increase of PS1 and the decrease of BDNF and TrkB induced by NaN_3 were most prominent in the CA1 region of the hippocampus, suggesting that the CA1 region may

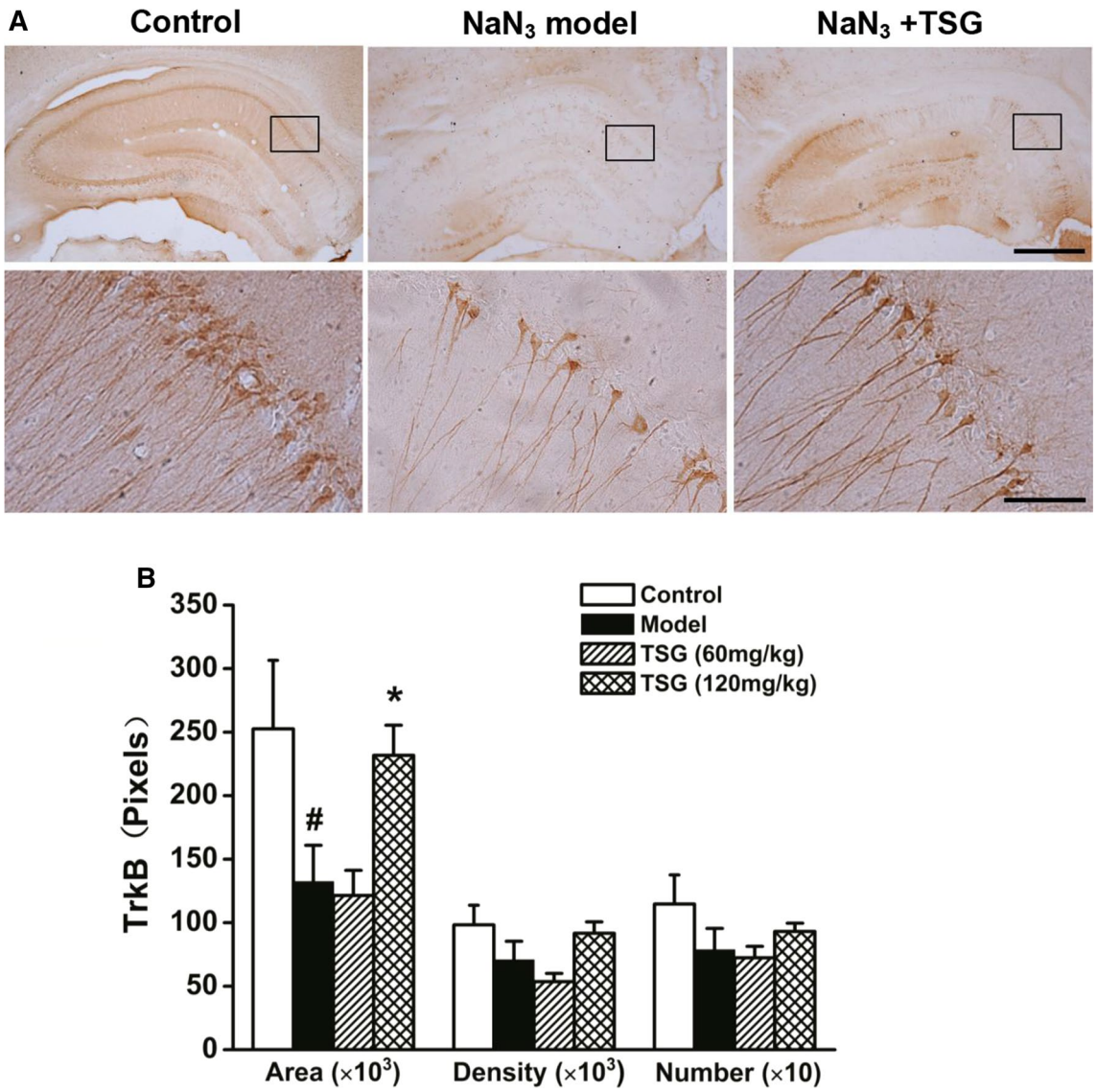

Fig. 9 Effects of TSG on TrkB expression in the hippocampal CA1 area of NaN_3-infused rats. **a** Representative images of immunohistochemistry for TrkB in the hippocampus. Scale bar in the upper row = 500 μm, in the lower row = 50 μm. **b** Image pixel analysis of the number, density and area of TrkB-positive neurons in hippocampal CA1 area. Data are expressed as the mean ± SEM from 9 sections of each group (3 sections per rat, 3 rats each group). $^{\#}P < 0.05$, NaN_3 model group versus the control group; * $P < 0.05$, TSG group versus the model group

be more vulnerable to mitochondrial dysfunction than other regions. We also found that TSG decreased PS1 expression and increased BDNF and TrkB expression in the CA1 area of the hippocampus, which may be beneficial for improving cognitive impairment in AD patients.

In the present study, the results showed that TSG at doses of 60 and 120 mg/kg dose-dependently increased the levels of NGF, BDNF and TrkB in the brain of NaN_3-infused rats. The effects of TSG elevating mitochondrial COX activity and decreasing the levels of Aβ, APP, BACE1 and PS1 did not show dose-dependence although both doses of TSG were efficacious. We speculate that the sensitivity of different targets to TSG may not be equal, and thus the effects of TSG at the doses of 60 and 120 mg/kg were different in each result.

In conclusion, the present study demonstrated that chronic infusion of NaN_3 induced a decrease in mitochondrial COX activity, an increase in Aβ content and the expression of APP, BACE1 and PS1, and a decline in the expression of neurotrophins in the brain of rats. Intragastrical administration of TSG elevated mitochondrial COX activity, reduced Aβ content by inhibiting the expression of APP, BACE1 and PS1, and elevated the levels of NGF, BDNF and its receptor TrkB in the brain of a mitochondria dysfunction rat model

induced by NaN_3 infusion. Because mitochondria dysfunction is an early event in the pathogenesis of AD, these results suggest that TSG may intervene in the early stage of AD pathogenesis, thus blocking or slowing the progression of AD.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this manuscript.

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