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Research report

Pharmacological activation of the Nrf2 pathway by 3H-1, 2-dithiole-3-thione is neuroprotective in a mouse model of Alzheimer disease

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ABSTRACT

Accumulating evidence suggests that oxidative stress induced by beta-amyloid (Aβ) is implicated in the pathological progression of Alzheimer's disease (AD). 3H-1,2-dithiole-3-thione (D3T), the simplest compound of the sulfur-containing dithiolethiones, has been proved to be a strongly active antioxidant factor by regulation of the nuclear factor E2-related factor 2 (Nrf2). Previous study reported that D3T confers protection to AD cell model in vitro, however, the neuroprotective effect of D3T in the AD mammalian model is unknown. In the present study, we aimed to evaluate the therapeutic potential of D3T in the Tg2576 AD mouse model and investigate the mechanisms underlying its beneficial effects. We showed that intraperitoneal administration of D3T significantly alleviated cognitive deficits in AD mice and dramatically decreased insoluble Aβ level and oxidative stress. Further mechanistic studies revealed that D3T significantly promoted hippocampal neurogenesis, and up-regulated levels of silent information regulator 1 (Sirt1), Nrf2 and heme oxygenase-1 (HO-1). Moreover, the positive effect of D3T on behavioral performance of AD mice was markedly attenuated by inhibition of the Sirt1/Nrf2 pathway by the antagonist EX527. In summary, our studies on a mouse AD model indicate that D3T could serve as a potential therapeutic agent for this devastating disease.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive memory loss, cognitive deficit and worsening activities of daily living, which results from impairment or loss of functional neurons [1,2]. The neuropathological hallmarks of AD include extracellular senile plaques and intracellular neurofibrillary tangles [3]. AD predominantly affects people over 65 years of age, and its morbidity is increasing. Today, someone develops AD every 66 s, and one new case of AD is expected to develop every 33 s, resulting in nearly 1 million new cases per year by 2050 according to the World Alzheimer report [4]. Despite the considerable advances in understanding the histopathologic features of AD, effective treatment to attenuate cognitive dysfunction remains to be extensively explored.

Numerous studies have shown that oxidative stress in hippocampus which can be triggered by Aβ plays a critical role in leading to pathological changes of AD, suggesting that antioxidant strategies may be of potential therapeutic avenue to alleviate overoxidation in hippocampus [5–7]. 3H-1,2-dithiole-3-thione (D3T), a cyclic sulfur-containing dithiolethione that is derived from cruciferous vegetables has been shown to potently induce production of antioxidants, thereby protecting cells from oxidative lesions, which is mainly mediated by activating the Nrf2 signaling pathway [8,9]. It has been shown that D3T could inhibit the formation of hyperoxidized peroxiredoxins following oxidative trauma in cortical neurons in vitro, protect SH-SY5Y cells against acrolein-induced neurocytotoxicity by up-regulation of cellular glutathione and protect retinal pigment epithelium cells against Ultra-violet radiation via activation of Nrf2 signaling [10–12]. In addition, recent studies also reported that D3T, which is a highly lipophilic agent capable of crossing the blood brain barrier when administered, could exert beneficial effect in neurological disease models including an animal model of ischemic stroke and a cell culture model of AD [11,13,14]. Thus, D3T is a potential novel therapeutic agent to treat neurodegenerative disorders.

Effects of D3T on AD mammalian model *in vivo* have not been

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evaluated previously. Therefore, we now report studies demonstrating neuroprotective effects of D3T in Tg2576 AD mouse model and provide preliminary insight into mechanisms by which D3T ameliorated cognitive dysfunction.

2. Experimental procedures

2.1. Animals

Tg2576 mice, on a C57BL/6 background, which harbors mutant human gene APPswe (Swedish mutations K670N/M671L) under the control of mouse prion protein promoter, were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Science (Beijing, China) as in our previous study. The experimental animal procedures were performed according to the Ethical Committee of Zhengzhou University. All mice were housed under a 12hr light/dark cycle and automatically maintained at 22–25 °C and humidity, given ad libitum access to food and water.

2.2. Drug administration and BrdU injection

D3T (Abcam, USA; purity > 98%) and the Sirt1 inhibitor EX527 (6-chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide, Sigma) were dissolved in dimethylsulfoxide (DMSO) in appropriate concentrations separately. Seven-month-old Tg2576 male mice were randomly divided into five groups: Wild type (WT) (n = 10), vehicle-treated AD (n = 15), D3T 10 mg/kg (body weight) treated AD (n = 10), D3T 20 mg/kg (body weight) treated AD (n = 15) and D3T (20 mg/kg) + EX527 (5 mg/kg) treated AD (n = 5). For WT group, we used age-matched non-transgenic C57BL/6 mice and intraperitoneally administrated 100 μL vehicle DMSO. For vehicle-treated AD group, we intraperitoneally administrated 100 μL vehicle DMSO. For D3T 10 mg and D3T 20 mg group, we intraperitoneally administrated 10 mg/kg (body weight) and 20 mg/kg (body weight) D3T solution separately. For D3T + EX527 group, we intraperitoneally administrated 20 mg/kg (body weight) D3T combined with 5 mg/kg (body weight) EX527 solution separately. All the mice were treated with drug or vehicle once per day for 60 days. No mouse died and there was no adverse effect during this study. Mice were subjected to intraperitoneal injection with 5-bromodeoxyuridine (BrdU) (50 mg/kg; Sigma) once a day from days 41 to 47 of drug administration.

2.3. Morris water maze test

On the day after the last drug administration, the Morris Water Maze (MWM) test was performed as previously described [15,16]. The MWM apparatus consists of a circular pool of 120 cm diameter and 40 cm height equipped with water (25 ± 1 °C) containing milk. A cylindrical escape platform diameter 10 cm and height 28 cm was positioned submerged 0.5–1 cm below the surface of the water. After training twice a day for six consecutive days, probe trial and navigation test were conducted on the seventh day. Swim paths of each mouse were monitored by an automated tracking computer system.

2.4. Tissue preparation

After MWM test, the mice were anesthetized with pentobarbital (50 mg/kg; Sigma) and immediately cardiac-perfused with 0.9% saline solution. For biochemical and molecular biological analysis, cerebral hemisphere or hippocampus was frozen in liquid nitrogen until use. For morphological analysis, cerebral hemisphere or hippocampus was fixed with 4% paraformaldehyde (PFA) for 24hr and incubated in 30% sucrose solution for 72 h, and then sequential 25 μm thickness coronal sections were cut on a cryostat and stored at −20 °C.

2.5. Aβ ELISA examination

Frozen hippocampus tissues were homogenized in T-PER regent containing protease inhibitors. After centrifugation at 14000*g* for 1 h (4 °C), the supernatant was collected to examine the soluble $A\beta_{40,42}$. Then the sediments were resuspended in 70% formic acid solution and centrifuged at 14000*g* for 1 h (4 °C), the supernatant was collected to detect the insoluble $A\beta_{40,42}$. $A\beta_{40,42}$ ELISA kits were used to exam the soluble and insoluble Aβ40 and Aβ42 levels and the procedures were in accordance with the user's manual.

2.6. Determination of oxidative stress

The frozen hippocampus tissues were homogenized in cold 0.9% saline solution, after centrifugation at 3500*g* for 15 min, the supernatant was collected to determine the oxidative stress markers including the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px), the levels of malonaldehyde (MDA) and glutathione (GSH) by using commercial kits (Jiancheng Bioengineering Inst., China). The procedures were according to the respective instructions.

2.7. Fluorescent immunohistochemical staining

The coronal sections including the dentate gyrus (DG) area were denatured in 2 N HCl for 30 min, rinsed in 100 mM boric acid (pH 8.0) for 10 min, washed with PBS at room temperature. And then the sections were incubated in PBS containing 10% normal donkey serum and 0.25% Triton X-100 for 1 h at room temperature. Afterward, sections were incubated with primary antibodies including rabbit anti-BrdU (Santa Cruz, USA; diluted in 1:250) and rat anti-NeuN (Millipore, USA; diluted in 1:1000) for 24 h at 4 °C and then incubated with the fluorescent probe-conjugated secondary antibodies including Cy3-conjugated donkey anti-rabbit (Jackson ImmunoResearch, USA; diluted in 1:500) and Alexa Fluor 647-conjugated donkey anti-rat (Jackson ImmunoResearch, USA; diluted in 1:500) for 1 h at room temperature. After washing with PBS and mounting on glass slides, sections were scanned by using a confocal microscope (Olympus, USA) to evaluate hippocampal neurogenesis.

2.8. Western blot analysis

Total proteins were extracted from the frozen hippocampus tissues by using RIPA lysis buffer containing PMSF. Protein content was quantified by using Nanodrop 2000 spectrophotometer (Thermo Fisher, USA). Equal amounts of denatured proteins (100 μg) were separated by SDS-PAGE. Then the protein was transferred to PVDF membrane. After blocking with 5% skim milk for 1 h, the membrane was incubated with diluted primary antibodies and HRP-conjugated secondary antibody separately. Immunoreactive bands on the membrane were detected by using the Chemidoc EQ system (Bio-Rad, USA). Primary antibodies as follows: anti-NEP (1:500, Santa cruz), anti-IDE (1:500, Santa cruz), anti-MMP-9 (1:500, Santa cruz), anti-BACE1 (1:500, Santa cruz), anti-PS1 (1:500, Millipore), anti-acetyl-Nrf2 (1:1000, Abbkine), anti-Nrf2 (1:2000, Abcam), anti-Sirt1 (1:1000, Abcam), anti-HO-1 (1:1000, Abcam) and anti-β-actin (1:5000, Abcam). β-actin was used as an internal control.

2.9. Statistical analysis

The data were analyzed by using the Graphpad Prism 5 software. All quantified data are presented as Mean ± Standard error of measurement (SEM). Pairwise comparisons were performed by using two-tailed student's *t*-test. For multiple comparisons, results were analyzed by using one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered to be statistically significant.