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Research report

Tripchlorolide improves age-associated cognitive deficits by reversing hippocampal synaptic plasticity impairment and NMDA receptor dysfunction in SAMP8 mice

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HIGHLIGHTS

- A novel extract of natural herbal, tripchlorolide has anti-dementia action.
- Long-term T_4 treatment prevents learning and memory loss in aged SAMP8 mice.
- T_4 ameliorates hippocampal LTP in aged SAMP8 mice.
- T_4 alleviates synaptic plasticity dysfunction of the hippocampus.
- T_4 intensified the signal pathway of CaMKII-CREB-BDNF in SAMP8 mice.

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ABSTRACT

Deficits in cognition and performance accompanying age-related neurodegenerative diseases such as Alzheimer's disease (AD) are closely associated with the impairment of synaptic plasticity. Here, using a mouse model of senescence-accelerated P8 (SAMP8), we reported the role of tripchlorolide (T_4), an extract of the natural herb *Tripterygium wilfordii Hook F*, in improving cognitive deficits and promoting the long-term potentiation (LTP) of hippocampal slices *via* the N-methyl-D-aspartate receptor (NMDAR)-dependent signaling pathway. Our results demonstrated that chronic administration of T_4 at low doses (0.25, 1.0, or 4.0 μg/kg per day, injected intraperitoneally for 75 days) significantly improved learning and memory function in aged SAMP8 mice, as indicated by a chain of behavioral tests including the Y-maze and Morris water maze. Additionally, T_4 reversed the impaired LTP in hippocampal CA1 regions of SAMP8 mice in a dose-dependent manner. Moreover, it upregulated the levels of phospho-NMDAR1, postsynaptic density-95 (PSD-95), phospho-calcium-calmodulin dependent kinase II (CaMKII), phospho-CREB and brain derived neurotrophic factor (BDNF) in the hippocampus. This indicates that T_4 prevents the impairment of NMDAR-mediated synaptic plasticity-related signal molecules. At optimal doses, T_4 did not show significant side-effects on blood counts, blood biochemical measures, or survival of the mice. This novel mechanism in reversing age-related synaptic dysfunction and NMDAR functional deficits suggests that T_4 can halt the manifestation of a key early-stage event in AD. With the consideration of SAMP8 mice as a model to develop therapeutic interventions for AD, our findings provide new insight into the clinical application of tripchlorolide in AD treatment.

Abbreviations: AD, Alzheimer's Disease; BDNF, brain-derived neurotrophic factor; CaMKII, calcium/calmodulin-dependent protein kinase II; CREB, cyclic AMP-response element binding protein; EPSPs, excitatory postsynaptic potentials; LTP, long-term potentiation; MWM, Morris water maze; NMDAR, N-methyl-D-aspartate receptor; PSD-95, postsynaptic density-95; SAMP8, senescence-accelerated mouse Prone 8 SAMR1; T_4, tripchlorolide.

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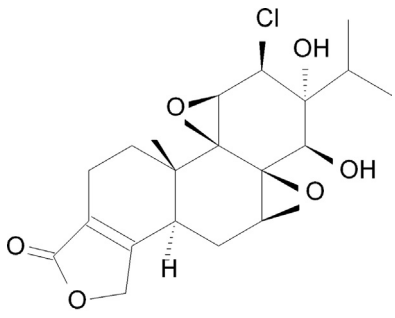
1. Introduction

Age is the most prominent risk factor in neurodegenerative diseases including Alzheimer's disease (AD). In aged adults, AD is the most common cause of dementia, which manifests the signs of memory loss, spatial disorientation, and weakness of intellectual capacity [1]. In fact, this age-related decline in cognitive function is associated with increased synaptic function change in brain.

Of all the animal AD research models, the senescence-accelerated prone-8 (SAMP8) displays irreversible advancing early senescence and exhibits cognitive impairment that reduces physical activity and erodes memory and thinking skills as seen in AD patient [2]. Interestingly, SAMP8 mice exhibit not only the cognitive deficits with the underlying mechanisms in synaptic, dendritic and memory alterations [3,4], but also pathological changes similar to those found in AD, such as the abnormal expression of amyloid precursor protein (APP) and amyloid-beta (Aβ) proteins, amyloid-like deposition in the brain, and increased physphorylation of Tau [5–8]. Different from other aged wild-type and single-transgenic (APP or PS1) and double-transgenic (APP + PS1) mice which present significant deficits in learning and long-term potentiation (LTP) properties that are not related with the presence of amyloid beta deposits [9], these mice have early amyloid accumulation in the hippocampus which then aggravates with age [6]. Both aging and Aβ decrease the neuronal plasticity by targeting N-methyl-D-aspartate receptor (NMDAR), which can adversely impair the cognitive performance. This impaired cognitive performance in aged SAMP8 mice can be improved by antisense to APP and antibody to reduce the level of Aβ in the brain [10–13]. In the early stage of AD, Aβ accumulation is also documented and found to be associated with synaptic dysfunction. Taken together, these findings suggest that the abnormal expression of Aβ contributes to the cognitive decline in aged SAMP8 mice and the animal model can be a useful tool to study the mechanisms underlying cognitive impairments and to explore the related drug treatments for AD [14].

Of all the candidate agents, tripchlorolide (designated as T_4, Fig. 1) is a novel extract from Chinese herb *Tripterygium wilfordii Hook F* (TWHF) that has been found to have potent anti-inflammatory and immunosuppressive functions and is widely used in China for treatment of rheumatoid arthritis [15,16]. Compared with other analogs, T_4 has a lower toxicity [17], and its lipophilic properties generated by chloridion modification and small molecular size (MW 397) most likely facilitate its passage through the blood–brain barrier. Many studies including epidemiological, basic and clinical research on AD have shown that nonsteroidal anti-inflammatory drug (NSAID) treatment reduces the risk of AD. As an extract from Chinese herb TWHF, T_4 has been found to have a significant neuroprotective effect in combatting inflammatory neurotoxicity induced by lipopolysaccharide-activated microglia [18]. It can protect neuronal cells not only from microglia-mediated Aβ neurotoxicity by inhibiting NF-κB and JNK signaling [19], but also directly protect against the neuronal apoptosis induced by Aβ, by regulating Wnt/β-catenin signaling [20]. Recently, in our studies on T_4, we also found that T_4 attenuates the secreted $A\beta_{1-42}$ and $A\beta_{1-40}$ in N2a/APP695 cells (paper unpublished, data not shown). These studies suggest that T_4 could be a modulator in the production of Aβ and a potential neurotrophic and neuroprotective agent in the treatment of AD.



Tripchlorolide

Fig. 1. The chemical structure of tripchlorolide (T_4).

Of the underlying mechanisms, synaptic loss and synaptic dysfunction are the most robust predictors of cognitive impairment in AD [21]. The pathogenesis of AD can be best explained by a loss of plasticity [22], which may have adverse dendritic ramifications, such as synaptic remodeling, long-term potentiation (LTP), axonal sprouting, neurite extension, synaptogenesis, and neurogenesis. To date, few studies have shown that NSAID could restore working memory deficit and decremental LTP and discussed the underlying mechanisms [23] and even scarce reports have focused on the role of T_4 in an age-related animal model with synaptic plasticity disruption, especially the effects of T_4 on LTP and synaptic plasticity-related proteins and the related signal activation. Here, we investigated the effects of long-term administration of T_4 on the improvement of learning and memory dysfunction in aged SAMP8 mice. Furthermore, we elucidated that T_4 that preserves the synaptic plasticity through regulating LTP and synaptic plasticity-related proteins including postsynaptic density-95 (PSD-95), and NMDAR. In addition, we observe the effect of long-term administration of T_4 at an optimal dose on the survival of mice and drug side-effects on blood counts and blood biochemical changes.

2. Materials and methods

2.1. Reagents

T_4 was obtained from Department of Pharmacology of Fudan University (Shanghai, China). The material was in the form of white needle-like crystals, with a melting point of 256–258 °C, a molecular weight of 397, and a purity of 98% by reverse phase high-pressure liquid chromatography (HPLC) evaluation.

2.2. Animals and treatment with

Virgin male SAMP8 and SAMR1 mice were generously provided by the Department of Laboratory Animal Science of Peking University. Each mouse was individually housed in a plastic cage with a constant temperature of 22 ± 0.5 °C and humidity of 60 ± 5% under a 12 h light–dark cycle (lights turned on at 6:00 a.m.). All mice received standard rodent diet and tap water *ad lib*. Any animals with gross defects (tumors outside trunk, motor incapacitation, or overt blindness) were excluded prior to the behavioral examination. All procedures used in these studies observed the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee of Fujian Medical University.

Mice were allowed 1 week to adapt to their environment after arrival. Before the being used for experiments, SAMP8 mice were tested in a Y-maze and then randomly allocated into four groups of 18–20, according to their Y-maze performance.

T_4 was prepared fresh and applied as a single daily injection at 9:00 a.m. It was first dissolved in dimethylsulphoxide (DMSO) and later diluted with 0.9% NaCl-physiological saline (N.S.) (DMSO < 0.05‰) and injected intraperitoneally. A single daily dose of 0.25, 1.0 and 4.0 μg/kg was respectively administered to SAMP8 mice aged 7.5 months for a total of 75 days. SAMP8 mice receiving vehicle (N.S.) were treated as a T_4-free control. The age-matched