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Dipotassium N-stearoyltyrosinate ameliorated pathological injuries in triple-transgenic mouse model of Alzheimer's disease

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ABSTRACT

Recently, anandamide (AEA) analogues have been well recognized for its potent neuroprotective effects in counteracting the deterioration of Alzheimer's disease (AD) brains through multiple pathological processes. In our previous studies, dipotassium N-stearoyltyrosinate (NSTK), an AEA analogue synthesized by our laboratory was reported to exert significant efficacy through multiple interventions. Within this study, the amyloid precursor protein $(APP)_{SWE}$/presenilin-1 $(PS1)_{M146V}/Tau_{P301L}$ mouse (3×Tg-AD) model was used to explore further the neuroprotective effects of NSTK and its underlying mechanisms. NSTK could increase spontaneous locomotor activity in the open field and low anxiety-like behavior in the elevated plus maze, and improve the spatial memory deficits in the Morris water maze. The biochemical analysis suggested that NSTK could decrease $A\beta_{42}$ deposition, abnormal tau aggregation, and the expressions of p-APP Thr668, PS1 and p-tau Ser202/Thr205 in the hippocampus of 3×Tg-AD mice. Consistently, NSTK could reduce the level of malondialdehyde, increase the activity of superoxide dismutase and catalase. Up-regulation of Bcl-2, and down-regulation of BAX, caspase-3 and inflammatory cytokines also occurred in the hippocampus of 3×Tg-AD mice after treatment with NSTK. Thus, NSTK could intervene in multiple pathological processes of AD and would be a drug candidate against AD.

1. Introduction

Alzheimer's disease (AD), a neurodegenerative disorder characterized by progressive cognitive dysfunction and behavioral impairment has become a major threat to human health. In AD brain, presenilin-1 (PS1) processes the amyloid precursor protein (APP) to generate amyloid (Aβ) peptides: as a matter of fact, clinical features of AD are manifested morphologically by excessive accumulation of extracellular aggregation of Aβ peptides in the form of amyloid plaques, and intracellular neurofibrillary tangles (NFTs) composed of perphosphorylated tau (1). Experimental evidence indicates that Aβ deposition and tau hyperphosphorylation trigger oxidative stress and inflammation, which lead to glial cell proliferation and eventually neuronal loss (2). Although numerous therapeutic agents aiming at the specific pathological process (3–5) have been developed, few of them are efficacious *in vivo* because of ignoring the integrity of pathogenesis and the relevance of multiple biological processes of AD and their applications in AD are limited. Therefore, it is imperative to find novel agents against AD via multiple pathways of intervention.

In damaged brain tissues, the endocannabinoid system (ECS) which consists of endocannabinoid (anandamide, AEA), its receptors and catabolic enzymes (e.g. fatty acid amide hydrolase, FAAH) regulates multiple pathological pathways in the central nervous system and has emerged as a potential target for the neuroprotection. It has been reported that the inhibitors of FAAH can decrease AEA hydrolysis and elevate AEA levels correspondingly, which are associated with anti-oxidative, anti-apoptotic and anti-inflammatory activities (6–8). Dipotassium N-stearoyltyrosinate (NSTK, Fig. 1), an AEA analog, was developed in our laboratory and is a promising neuroprotective candidate against stroke currently under preclinical studies in China. NSTK could ameliorate cognitive dysfunction induced by chronic cerebral hypoperfusion/global cerebral ischemia in rats/

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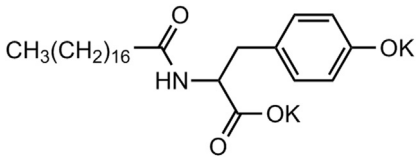

Fig. 1. Chemical structure of NSTK.

gerbils (10,11). We found the mechanisms of NSTK against stroke involve the inhibition of FAAH and the subsequent indirect activation of cannabinoid receptors (12), which finally improves inflammation, oxidative stress and glutamate-induced toxicity and cell viability (9–11). The above researches showed that NSTK has good neuroprotective effects through ECS-mediated multiple pathways.

Since inflammation, oxidative stress and glutamate-induced apoptosis are the common pathological characteristics in stroke as well as in AD (13–15) and NTSK had the potential to improve the pathological processes in cerebral ischemia model, NSTK might be also effective in AD treatment. Indeed, we have demonstrated the effects of NSTK against Aβ-induced toxicity on primary cortical neurons (12). It is interesting to observe the anti-AD effect of NSTK based on the animal model, especially on the triple-transgenic mouse model of AD (3×Tg-AD mice) which over-expresses human APP, PS1 and tau mutations. Since 3×Tg-AD mice not only progressively develop the typical pathological features and show behavioral impairments, but also mimic many aspects of human AD (16), it is of great clinical significance to validate the efficacy of NSTK on the model of AD.

In the present study, 3×Tg-AD mice were used to confirm the efficacy and mechanisms of NSTK in the treatment of AD. We first observed the animal behaviors as well as typical pathological features of the mice to evaluate the efficacy of NSTK against AD. Then, the key factors related to oxidative stress, inflammation and apoptosis in the transgenic mice were investigated to confirm the underlying mechanism of NSTK. In accordance with our previous observations in neuron (12), NSTK obviously improves behaviors and pathological features in 3×Tg-AD mice. Moreover, we found the mechanisms involved not only the improvement of glutamate-induced toxicity, which we have confirmed recently (9), but the decrease of inflammatory response, oxidative stress and the inhibition of apoptosis via ECS indirect activation.

2. Materials and methods

2.1. Chemicals

NSTK with purity over 98% was prepared in our laboratory and its structure was confirmed by ^{1}H NMR and ^{13}C NMR. NSTK and Piracetam purchased from Sigma Chemical Co. (St. Louis, MO, USA) were dissolved in sterilized water and stored at −20 °C.

2.2. Animals

The 3×Tg-AD mice were obtained by crossing heterozygous APPswe/PS1dE9 double transgenic mice (Jackson Laboratory, Bar Harbor, ME, USA) with heterozygous P301L tau transgenic mice (Taconic Labs, Germantown, N.Y.). The wild male C57BL/6J mice (Shanghai SLAC Laboratory Animal CO., Ltd, Shanghai, China) and 3×Tg-AD mice were maintained in a controlled environment at 25 ± 1 °C with a 12/12 h light-dark cycle. The experimental protocols were performed according to the Guidelines for Animal Experimentation of Shanghai Jiao Tong University.

2.3. Experimental treatment

50 male 3×Tg-AD mice (9-month-old at the start of the study) were randomly divided into five groups (each n = 10): the 3×Tg-AD group, three groups of 3×Tg-AD mice treated with 15, 30 and 45 mg/kg NSTK respectively, and the 3×Tg-AD group treated with piracetam (100 mg/kg) as the positive control. Each mouse was administrated orally in a volume of 0.1 mL per 10 g weight for 2 months. The wild mice were used for the control group (n = 10). Experimental groups and the wild group received an equal volume of NSTK and distilled water, respectively. The animals were behaviorally tested after 2 months of treatment. The behavioral tests were carried out in the following order: open field testing (OFT), elevated plus-maze (EPM), Morris water maze (MWM). The animals were sacrificed and the brains were removed from the skulls after the animal experiment. Half of the brain was used for immunofluorescence, and the rest for western blotting and enzyme-linked immunosorbent assay (ELISA). Since the hippocampus is the major part of learning and memory function, we used the tissue homogenates of the hippocampus for western blotting and ELISA in this study.

2.4. OFT

The OFT was used to assess general locomotor activity. Each mouse was placed in the center of the open field apparatus (50 × 50 × 38 cm) equipped with a video-tracking system and allowed to explore the apparatus for 5 min with the experimenter out of view. The total distance moved in the arena was recorded.

2.5. EPM

The apparatus (50 cm height from floor) is consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm) and is used to measure anxiety-like behaviors of the mouse. Each mouse was placed in the central section facing an open arm and was allowed to explore the maze for 5 min with the experimenter out of view. The time spent in the open arms was recorded using a video camera.

2.6. MWM

The MWM is consisted of a circular pool (1.8 m in diameter) filled with water (24 ± 1 °C) and is surrounded with curtains to avoid environmental distraction. It was used to assess the spatial learning and memory. The target quadrant contained an escape platform (9 cm in diameter) which was submerged 1 cm below the surface of water in the center of one quadrant. During the training, each mouse was given 4 trials per day for 5 consecutive days. During each trial, the mouse was placed into the pool facing the wall at one random quadrant and allowed 60 s to locate the platform. Any animal who did not locate the platform was guided and placed on the platform for 30 s. On the 6th day, probe trials were carried out in which the platform was removed and the mice were given 60 s to search for the target quadrant. The escape latency, the time spent in the target quadrant and searching strategy were recorded by the video-tracking system.

2.7. Immunohistochemistry

The 3×Tg-AD mice were anesthetized with pentobarbital, perfused with saline, and then perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. The brains were removed from the skulls, fixed in 4% paraformaldehyde for 24 h and then transferred into PBS containing 30% sucrose. Each brain was

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