Neuroscience Letters 610 (2016) 117–122

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Estrogen receptor-mediated effect of δ-tocotrienol prevents neurotoxicity and motor deficit in the MPTP mouse model of Parkinson's disease

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HIGHLIGHTS

- Oral administration of δT3 may be useful in the treatment of MPTP-induced PD model.
- ERβ may be a candidate target for the neuroprotection activity of δT3.
- δT3 may prevent motor deficit in the MPTP mouse model of Parkinson's disease.

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 20 October 2015
Accepted 24 October 2015
Available online 30 October 2015

Keywords:
Vitamin E
Tocotrienol
Parkinson's disease
Estrogen receptor
MPTP

ABSTRACT

Neuroprotection following signal transduction has been investigated recently as a strategy for Parkinson's disease (PD) therapy. While oxidative stress is important in the pathogenesis of PD, neuroprotection using antioxidants such as α-tocopherol have not been successful. δ-tocotrienol (δT3), a member of the vitamin E family, has received attention because of activities other than its antioxidative effects. In the present study, we examined the estrogen receptor-β (ERβ)-mediated neuroprotective effects of δT3 in a mouse model of PD. ERβ is expressed in neuronal cells, including dopaminergic neurons in the substantia nigra. Daily forced oral administration of δT3 inhibited the loss of dopaminergic neurons in the substantia nigra. In addition, the ER inhibitor tamoxifen canceled the neuroprotective effects of δT3. Moreover, δT3 administration improved the performance of the PD mice in the wheel running activity, while tamoxifen inhibited this improved performance. These results suggest that the oral administration of δT3 may be useful in the treatment of PD patients, and ERβ may be a candidate target for the neuroprotection activity of δT3.

1. Introduction

Parkinson's disease (PD) is a common neurological disease caused by the degeneration of dopaminergic neurons in the substantia nigra, with the subsequent loss of their terminals in the striatum [1]. The ensuing loss of the neurotransmitter dopamine causes most of the debilitating motor disturbances, such as akinesia, rigidity, and tremor. Although current PD medications treat motor symptoms of the disease without halting or retarding the degeneration of dopaminergic neurons, there has been considerable interest in neuroprotection as a therapeutic strategy for PD in recent years [2,3]. Therefore, several drugs have been proposed and investigated as candidate agents for PD [4–6]. Oxidative stress contributes, at least in part, to the pathogenic cascade leading to neurodegeneration of dopaminergic neurons in PD [1,4]. Therefore, antioxidants have been proposed as potential candidates for neuroprotective therapy in PD. However, antioxidative therapy for PD using exogenous antioxidants has not been successful in the clinic setting thus far [7].

Abbreviations: SDS, sodium dodecyl sulfate; DAB, 3,3'-diaminobenzidine,tetrahydrochloride.

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http://dx.doi.org/10.1016/j.neulet.2015.10.062
0304-3940/

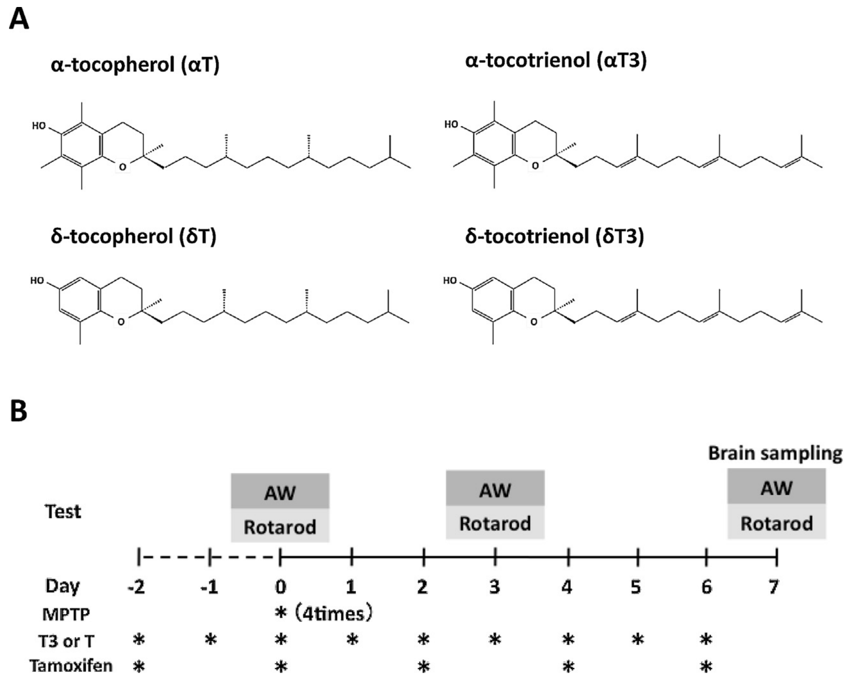

Fig. 1. Structures of compounds used in this study and administration protocol.
(A) Structure of α-tocopherol (αT), δ-tocopherol (δT), α-tocotrienol (αT3) and δ-tocotrienol (δT3). (B) Experimental protocol. MPTP was injected intraperitoneally 4 times at day 0, and oral administration of αT, δT, αT3 and δT3 was carried out every day from day -2 to day 6. Tamoxifen was injected intraperitoneally every 2 days from day -2 to day 6.

α-Tocopherol (αT), a member of the vitamin E family, is a well-known antioxidant and has been investigated in a mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) model of PD [8,9]. However, most studies failed to show improvement in the neurotoxicity and motor deficit in PD patients and the animal model of PD.

Previously, we reported that a low concentration of δ-tocotrienol (δT3), also a member of the vitamin E family, shows neuroprotective effects mediated by the estrogen receptor β (ERβ) –PI3K/Akt pathway in a PD model of SH-SY5Y cells [10]. In this in vitro cellular PD model, δT3 protected cells through signal transduction rather than through antioxidative effects against 1-methyl-4-phenylpyridinium ion (MPP^+)-induced cytotoxicity [10]. However, the in vivo effect of δT3 in MPTP-induced neurotoxicity is not well understood. Therefore, in this study, we investigated the estrogen receptor-mediated protective effect of oral δT3 administration in the MPTP mouse PD model.

2. Materials and methods

2.1. Reagents and antibodies

Four types of compounds from the vitamin E family were prepared (Fig. 1A). α-tocotrienol (αT3) and δT3 were provided by the Eisai Food Chemical Co., Ltd. (Tokyo, Japan). MPTP, αT, and δ-tocopherol (δT) were purchased from Sigma (St. Louis, MO). Tamoxifen was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Anti-ERβ antibody and anti-β actin antibody were purchased from Cell Signaling Technology (Danvers, MA). Anti ER-α antibody and anti-tyrosine hydroxylase (TH) antibody were obtained from Santa Cruz Biotechnology (Dallas, TX) and Chemicon International Inc. (Billerica, MA), respectively.

2.2. Animal model and behavioral testing

C57BL/6 mice were obtained from CLEA Japan, Inc, (Tokyo, Japan) and housed in an air-conditioned room (25 °C and approximately 50% relative humidity) with free access to food and water. The mice (30–35 g) were treated in accordance with the Guideline for Animal Experimentation of Tottori University (No.14-Y-03). The experimental procedure is shown in Fig. 1B. δT3 was dissolved in ethanol to a concentration of 100 mM, and then diluted in water to a final concentration of 1 mM. δT3 (100 μg/kg body weight) or vehicle was administered every day (from the day -2 to the day 6) using oral gavage needles (Fig. 1B). Tamoxifen (10 μg/kg body weight) was administered intraperitoneally every 2 days (Fig. 1). MPTP was dissolved in saline (5 mg/ml) and 20 mg/kg body weight of MPTP or vehicle was injected intraperitoneally 4 times at 1 h intervals.

For behavioral assessment, wheel running activity (WRA) was examined using an MK-750PC (Muromachi, Japan), and running activity was converted to revolutions per hour. Rotarod disability was examined using the MK-630B treadmill (Muromachi, Japan). The drum was slowly accelerated from a speed 4 to 40 rpm over the duration of 360 s. The latency to fall off the rotarod within this period was recorded.

2.3. Immunoblots

For immunoblots, mice were decapitated under deep anesthesia (pentobarbital, 50 mg/kg, i.p.) and brain tissues were collected. Brains were separated into five parts (cerebral cortex, striatum, substantia nigra, cerebellum, and olfactory bulb), and the tissues were lysed in SDS sample buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 2 mM ethylenediaminetetraacetic acid). Aliquots (20 μg) were separated on the basis of molecular size on 10% polyacrylamide gels, transferred onto polyvinylidene difluoride membranes (Hybond-P; GE Healthcare, Buckinghamshire, UK), and hybridized with the required antibody in PBS-Tween 20 at room temperature for 1 h. The immunoreactive signal was detected using horseradish peroxidase-linked anti-rabbit or mouse IgG and ECL detection reagents (GE Healthcare). Protein content of each sample was mea-