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Research report

α-Tocopherol administration produces an antidepressant-like effect in predictive animal models of depression

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ABSTRACT

This study investigated the antidepressant potential of α-tocopherol, the most active and abundant form of vitamin E, in the forced swim test (FST) and tail suspension test (TST). The acute oral treatment with α-tocopherol at the doses of 30 and 100 mg/kg reduced the immobility time in the FST and in the TST. A single i.c.v. administration of α-tocopheryl phosphate, a water-soluble analogue of α-tocopherol, also reduced the immobility time in the FST (0.1 and 1 nmol/site) and in the TST (0.1 nmol/site). In addition, the long-term treatment (28 days) with α-tocopherol (10 mg/kg, p.o.) significantly reduced the immobility time in the FST. Moreover, a subeffective dose of α-T (10 mg/kg, p.o.) potentiated the effect of fluoxetine (10 mg/kg, p.o.) in the FST. The long-term treatment with α-T was able to increase the glutathione (GSH) antioxidant defense system, while the acute treatment was not. The long-term treatment with α-tocopherol (10 mg/kg) increased the GSH levels in the hippocampus and in the prefrontal cortex and increased the glutathione peroxidase and glutathione reductase activity in the hippocampus (10 mg/kg) and in the prefrontal cortex (10–100 mg/kg). The long-term treatment with fluoxetine (10 mg/kg, p.o.), a positive control, was also able to increase the GSH levels in the hippocampus, but failed to alter the activity of both enzymes. Besides the specific antidepressant-like effect, long-term, but not the acute treatment with α-T, especially in the doses that produced an antidepressant-like effect (10 mg/kg), improved the antioxidant defenses in the mouse hippocampus and prefrontal cortex, two structures closely implicated in the pathophysiology of depression.

1. Introduction

Vitamin E is an essential micronutrient present at high concentrations in green leafy vegetables, vegetable oils, nuts and seeds [14,52]. Natural vitamin E includes two groups of closely related compounds, the tocopherols and tocotrienols, each with four analogues [43]. α-Tocopherol (α-T), the most active and abundant form of vitamin E in human plasma, is the major fat-soluble chain-breaking antioxidant that acts preventing the propagation of free radical reactions in membranes and lipoproteins [1,51]. This compound also exists in the natural water-soluble form α-tocopheryl phosphate, esterified with phosphoric acid, which makes this form of vitamin E an ideal candidate for a number of cellular functions, such as oxidant-protected intracellular transport, enzymatic regulation, and cell signaling [25,35,38]. Moreover, α-T is part of a complex non-enzymatic antioxidant defense system, which includes glutathione and ascorbic acid [11]. Besides its antioxidant activity, several preclinical and clinical studies have shown that α-T has pronounced anti-inflammatory and anti-atherogenic properties [20,42,46].

Depression is a chronic, recurring and potentially life threatening mood disorder that affects up to 20% of the world population [7]. It has profound social and economic consequences, with individuals often experiencing high rates of complicating comorbidities and mortality, like cardiovascular diseases and suicidality, and significant personal and societal costs due to decreased work productivity and utilization of health care services [36]. Current pharmacological treatment of depression is predominantly focused on the enhance-

Abbreviations: ANOVA, analysis of variance; DTNB, 5,5-dithiobis-(2-nitrobenzoic acid); FST, forced swimming test; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; p.o., per os; NADPH, nicotinamide adenine dinucleotide phosphate; TST, tail suspension test; α-T, α-tocoferol; α-TP, α-tocopheryl phosphate.

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ment of monoaminergic neurotransmission, and despite the large use in clinic, it still provides challenges with regard to the onset of action, lack of efficacy and occurrence of side effects [37,58]. Therefore, the identification of alternative therapeutic tools for the treatment of depression is still needed.

Oxidative stress mechanisms have been implicated in the pathophysiology of psychiatric disorders [8,40]. Oxidative stress occurs when redox homeostasis is challenged by free radicals, due to either their overproduction or deficiencies in antioxidant defenses [45]. The brain is particularly vulnerable to oxidative damage, since it has a comparatively high oxygen utilization, modest antioxidant defenses, a lipid-rich constitution that provides substrates for oxidation, the presence of redox-catalytic metals such as iron and copper, and neurotransmitters with reducing potential [27,55]. In brain, free radicals, highly unstable molecules with unpaired electrons, have potential to damage cellular proteins, lipids, carbohydrates and nucleic acids, leading to neurodegeneration [55]. This vulnerability of the brain and the association between neurodegenerative changes and psychiatric disorders suggest that oxidative damage mechanisms may be implicated in the pathogenesis of these disorders, and that the antioxidant supplementation may be a novel target in its treatment [6].

Enhanced oxidative stress or defective antioxidant defenses are also related to major depression [53,54]. A significant positive correlation was found between oxidative stress index and the Hamilton Depression Rating Scale [59] and studies have shown that depressive patients have low serum activity of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, as well as higher levels of lipoperoxidation products as malondialdehyde and lipid hydroperoxide, in comparison with the healthy controls [8,31,40,52]. Moreover, studies have provided evidence for the antioxidant effects of selective serotonin reuptake inhibitors, by demonstrating reversal of antioxidant and oxidative disturbances in depressive patients after antidepressant treatment [8,29,31]. In addition, it has been shown that major depression is accompanied by a significant reduction in serum vitamin E concentrations [33] and that α-T levels are inversely related to the Beck Depression Inventory scores [39]. These findings are in agreement with preclinical studies that showed that the treatment with α-T attenuates the depressive-like symptoms induced by lipopolysaccharide administration in mice [4,26], but no study was conduced in specific animal models of depression.

Taking into account the lack of preclinical studies dealing with the antidepressant-like effects of α-T, the present work was aimed at investigating the effect of acute treatment of mice with α-T (administered orally and centrally) in the forced swimming test (FST) and in the tail suspension test (TST), two widely used behavioral tests that predict the efficacy of antidepressant treatments [10]. Moreover, we sought to investigate the effect of long-term treatment with α-T in the FST, the most used predictive model of depression. Considering the well-established role of vitamin E as an antioxidant, this study also investigated the effect of acute and long-term treatment with α-T in the glutathione antioxidant defense system in the hippocampus and prefrontal cortex of mice, two structures closely implicated in the pathophysiology of depression [37].

2. Methods

2.1. Animals

Female Swiss mice, weighing 30–40 g (50–70 days old) were maintained at 22–24 °C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 7:00 h). This study was performed in female mice, since several studies have shown that the prevalence of depression is about two fold higher in women than in men [58]. All experiments were carried out between 9:00 and 16:00 h, with each animal used only once. The experiments were performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) and were carried out after approval of the protocol by the Ethics Committee of the Institution and all efforts were made to minimize animal suffering.

2.2. Drugs and treatment

For the behavioral studies, the following drugs were used: (±)-α-tocopheryl phosphate disodium salt (α-TP), DL-all-*rac*-α-tocopherol (α-T) and fluoxetine. For the neurochemical determinations, the following chemicals were used: nicotinamide adenine dinucleotide phosphate (reduced form; NADPH), tertbutylhydroperoxide, oxidized (GSSG) and reduced (GSH) glutathione, glutathione reductase (GR), 5,5dithiobis-(2-nitrobenzoic acid) (DTNB) and bovine serum albumin (Sigma Chemical Co, USA). For acute treatment, α-T was dissolved in mineral oil with 10% of ethanol and α-TP (administered by i.c.v. route) was dissolved in saline. For long-term treatment, α-T was dissolved in corn oil with 10% of ethanol. Fluoxetine was dissolved in distilled water. Control animals received appropriate vehicle (acute treatment: mineral oil with 10% of ethanol in the experiments with α-T, saline in the experiments with α-TP and distilled water in the experiments with fluoxetine; long-term treatment: corn oil with 10% of ethanol in the experiments with α-T and distilled water in the experiments with fluoxetine).

In order to investigate the possible antidepressant-like effect produced by an oral administration (p.o.) of α-T, mice were treated with α-T (10, 30, 100 and 300 mg/kg) 60 min before the FST, TST or open-field test. Alternatively, in order to confirm that the effect produced by the p.o. administration of α-T is due to its entering into the central nervous system, α-TP, a water-soluble natural form of vitamin E, was directly administered through i.c.v. injection (dose range: 0.1–10 nmol/mice) 15 min before the animals being submitted to the same behavioral tests. An independent group of mice were treated acutely with α-T (10, 30, 100 mg/kg), fluoxetine (10 mg/kg, p.o.) or vehicle. After 60 min mice were killed by decapitation and the hippocampi and prefrontal cortices were removed for neurochemical analysis.

To investigate the effect of long-term administration of α-T in the FST, animals received α-T (10–100 mg/kg, p.o.) or fluoxetine (10 mg/kg, p.o., positive control) for 28 days. Twenty-four hours after the last treatment, the FST or the open-field test were carried out. Twenty-four hours after these tests, animals were killed by decapitation and the hippocampi and prefrontal cortices were removed for neurochemical analysis.

In a separate set of experiments, an independent group of animals were pretreated with a subeffective dose of α-T (10 mg/kg, p.o.) or vehicle (mineral oil with 10% of ethanol) and immediately after, they received the antidepressant fluoxetine (10 mg/kg, p.o., serotonin reuptake inhibitor) or vehicle (distilled water). After 60 min, the open-field test or the FST were carried out. The dose of fluoxetine was selected based on a previous study of our group [15].

2.3. Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed under ether anesthesia as described previously [3,30]. Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, which was linked to a 25 μl Hamilton syringe, was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse. A volume of 5 μl was then administered in the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1 mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, the brains were dissected and examined macroscopically after the test.

2.4. Forced swimming test

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C; the total duration of immobility during a 6-min test was scored as described previously [15,30]. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect [41].

2.5. Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. [49]. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period [32,44].

2.6. Open-field test

In order to rule out an interference of the locomotor activity in the interpretation of the results obtained in FST and in TST, the ambulatory behavior was assessed in an open-field test as described previously [32,44]. The apparatus consisted of a wooden box measuring 40 cm × 60 cm × 50 cm high. The floor of the arena was divided into