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Antidepressant-like effect of α-tocopherol in a mouse model of depressive-like behavior induced by TNF-α

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ABSTRACT

Taking into account that pro-inflammatory cytokines and oxidative and nitrosative stress are implicated in the pathogenesis of depression and that α-tocopherol has antidepressant, anti-inflammatory and antioxidant properties, this study investigated the ability of α-tocopherol to abolish the depressive-like behavior induced by i.c.v. administration of TNF-α in the mouse TST. Additionally, we investigated the occurrence of changes in the levels of Bcl2 and Bax and phosphorylation of GSK-3β (Ser9) in the hippocampus of mice. The administration of TNF-α (0.001 fg/site, i.c.v.) increased the immobility time in the TST, which was prevented by the administration of α-tocopherol at the doses of 10, 30 and 100 mg/kg (p.o.). Subeffective doses of α-tocopherol (10 mg/kg, p.o.) and/or the antidepressants fluoxetine (5 mg/kg, p.o.), imipramine (0.1 mg/kg, p.o.) and bupropion (1 mg/kg, p.o.), the NMDA receptor antagonist MK-801 (0.001 mg/kg, p.o.) or the neuronal nitric oxide synthase inhibitor 7-nitroindazole (25 mg/kg, i.p.) prevented the depressive-like effect induced by TNF-α. None of the treatments altered the locomotor activity of mice. Treatment with TNF-α and/or α-tocopherol did not alter the levels of Bax and Bcl2 or the phosphorylation of GSK-3β in the hippocampus of mice. Together, our results show a synergistic antidepressant-like effect of α-tocopherol with antidepressants against the depressive-like behavior induced by an inflammatory insult, suggesting that this vitamin may be useful to optimize conventional pharmacotherapy of depression, including depressive states associated with inflammatory conditions.

1. Introduction

Depression is a neuropsychiatric disorder that has a high rate of comorbidity and mortality, beyond providing high social and personal costs (Simon, 2003). Among the various factors involved in the pathophysiology of depression, it is known that changes in inflammatory pathways are correlated with this mood disorder (Dantzer et al., 2008). The relationship between depression and inflammation was established based on numerous studies and meta-analysis showing that depressive patients have increased inflammatory markers such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) (Dowlati et al., 2010; Howren et al., 2009). Besides, the presence of some inflammatory diseases, such as inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and neuro-inflammatory disorders, is believed to increase the risk for the development of depression (Graff et al., 2009). Corroborating the inflammatory hypothesis of depression, it was demonstrated that patients with cancer or chronic hepatitis C, who are treated with inflammatory cytokines, have a higher risk of depression (Bonaccorso et al., 2001; Capuron et al., 2000). Similarly, it has been shown that the administration of proinflammatory cytokines, such as interferon (IFN)-α and TNF-α induces a depressive-like behavior in mice (Kaster et al., 2012; Ping et al., 2012).

It is known that an excessive activation of inflammatory pathways in the brain can lead to many consequences which are also seen in depression, such as increased oxidative and nitrosative stress, decreased neurotrophic factors, glutamatergic excitotoxicity and loss of glial elements (Maes, 2008; McNally et al., 2008). Moreover, an excess of inflammatory cytokines in the central nervous system (CNS) decreases serotonin production, enhancing the kynurenine pathway. As a result of overactivation of this pathway, there is an increased level of metabolites that include quinolinic acid, an N-methyl-D-aspartate (NMDA) receptor agonist (subtype of ionotropic glutamate receptor), which may contribute to elevate the risk of glutamatergic excitotoxicity (McNally et al., 2008; Myint et al., 2012).

Inflammation also can influence neuronal function because inflammatory cytokines such as TNF-α and IFN-γ may alter metabolic processes, oxidative and nitrosative stress, excitotoxicity and apoptosis (Hayley et al., 2005). TNF-α, a cytokine used in this study, has been shown to

Abbreviations: CNS, central nervous system; FST, forced swimming test; GSK-3, glycogen synthase kinase-3; IFN, interferon; IL, interleukin; i.c.v., intracerebroventricular; i.p., intraperitoneal; NMDA, N-methyl-D-aspartate; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; O.D., optical density; p.o., per oral; TST, tail suspension test; TNF-α, tumor necrosis factor-α.

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exacerbate cell death, and to impair neurogenesis, leading to neurodegeneration (Patel et al., 2006; Viviani et al., 2004).

It is important to be mentioned that decreased hippocampal volume, commonly observed in depressive patients, is closely related to reduced neurogenesis and/or increased neuronal/glial death, a scenario also observed in the presence of high levels of inflammatory cytokines (MacQueen et al., 2003; Malykhin et al., 2010). Various proteins are involved in the cell death process, among which stands out the Bcl2 family. This set of proteins integrates death and/or cell survival signals generated within or outside the cell, regulating cell death induced by apoptosis (Borner, 2003). Bcl2 family is divided into two classes: pro-apoptotic members such as Bax and Bak and antiapoptotic members such as Bcl2 and Bcl-xL (Antonsson, 2001; Borner, 2003). Another protein that has been shown to be involved in the modulation of apoptosis is the enzyme glycogen synthase kinase-3β (GSK-3β), that is inhibited by phosphorylation at the serine 9 residue (Beaulieu et al., 2009). Activation of GSK-3β has been shown to promote apoptosis in a wide variety of conditions, including neurodegenerative diseases and depression (Jope and Johnson, 2004; Maes et al., 2012). Besides its involvement in apoptosis, GSK-3β is also important in regulating the inflammatory response (Cortes-Vieyra et al., 2012). Additionally, some studies have shown an association between GSK-3β and animal models of depression, and inhibition of this protein is associated, at least partly, with the therapeutic action of antidepressant agents (Beaulieu et al., 2008; Budni et al., 2011).

The oxidative and nitrosative stress has also been associated with the pathophysiology of depression. It may result in lipid peroxidation, protein and DNA oxidation, membrane disruption, cell permeation, apoptosis, modifying the cellular signaling, differentiation, and proliferation, increasing the inflammation and producing deleterious cellular effects, contributing to the development or severity of depression (Behr et al., 2012; Maes et al., 2011; Ng et al., 2008; Sarandol et al., 2007). Furthermore, both inflammation and oxidative and nitrosative stress may contribute to neurodegeneration processes, increasing the neuronal apoptosis and reducing the neurogenesis and the production of neurotrophic factors, events that together may contribute to the phenotype of depression (Kubera et al., 2011; Leonard and Maes, 2012; Moylan et al., 2013). Interestingly, some antidepressants have antioxidant and anti-inflammatory effects (Abdel-Salam et al., 2004; Behr et al., 2012; Zafir et al., 2009) and some antioxidants (e.g. N-acetyl-L-cysteine, green tea polyphenols, ascorbic acid) have been reported to exert antidepressant-like effects (Ferreira et al., 2008; Moretti et al., 2011, 2012; Zhu et al., 2012).

Besides the involvement of inflammation and oxidative and nitrosative stress, it is reported that the hyperactivity of glutamatergic system, particularly through NMDA receptors, is also involved in the pathophysiology of depression (Skolnick, 1999; Zarate et al., 2010). This hypothesis is reinforced by preclinical and clinical studies showing that NMDA receptor antagonists have antidepressant activity (Garcia et al., 2008; Sanacora et al., 2012). Furthermore, NMDA receptor antagonists such as MK-801 and ketamine were also reported to possess anti-inflammatory action, a property that may contribute to their antidepressant effect (Esposito et al., 2011; Wu et al., 2012). A consequence of the activation of NMDA receptors is the stimulation of neuronal nitric oxide synthase (nNOS), which converts L-arginine to nitric oxide (NO) and L-citrulline (Calabrese et al., 2007; Steinert et al., 2010). NO has been considered a neurotransmitter substance, but it is also involved in CNS disorders, including depression, which has been associated with increased levels of NO in the brain (Dhir and Kulkarni, 2011).

Although the current treatment for depression is usually safe, it is still not ideal, since it has some limitations, in addition to presenting several side effects (Nemeroff and Owens, 2002). Given the limited effectiveness of current treatment options, our group has studied the antidepressant-like effect of some nutrients (Budni et al., 2011; Moretti et al., 2013), aiming at improving the treatment of depression.

Vitamin E is a fat soluble vitamin that includes eight different chemical analogs present in foods: alpha (α), beta (β), gamma (γ) and delta (δ)-tocopherol and α-, β-, γ- and δ-tocotrienol, and of these, α-tocopherol is the most abundant in foods (Traber, 2007). α-Tocopherol has several functions for humans, including antioxidant, anti-inflammatory, anticancer and antiatherogenic activities, direct effects on enzymatic activity and regulation in the transcription of some genes (Schneider, 2005; Tucker and Townsend, 2005). Furthermore, besides α-tocopherol has been shown to exhibit antidepressant properties in preclinical studies (Lobato et al., 2010), clinical studies demonstrated that patients with depression had lower serum (Maes et al., 2000) or plasma (Owen et al., 2005) levels of vitamin E.

Considering this background regarding the involvement of inflammation and oxidative and nitrosative stress in the pathophysiology of depression and taking into account the potential anti-inflammatory and antioxidant of α-tocopherol, in the present study we evaluated the antidepressant-like effect of α-tocopherol in an animal model of depressive-like behavior induced by TNF-α. Moreover we evaluated the potential synergistic effect of the combined administration of subeffective doses of α-tocopherol and conventional antidepressants, as well as the combined effect of subeffective doses of α-tocopherol and the NMDA receptor antagonist MK-801 or the neuronal nitric oxide synthase inhibitor 7-nitroindazole in the model of depressive-like behavior induced by TNF-α.

2. Materials and methods

2.1. Animals

The experiments were conducted using female Swiss mice (45–55 days old, weighing 30–45 g), maintained at 20–22 °C with free access to water and food, under a 12:12 h light/dark cycle (lights on at 7:00 a.m.). All behavioral tests were carried out between 9:00 a.m. and 04:00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals and the experiments were performed after approval of the protocol by the Ethics Committee of the Institution. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs and treatment

The following drugs were used: DL-all-rac-α-tocopherol (10, 30 and 100 mg/kg), fluoxetine (5 mg/kg; selective serotonin reuptake inhibitor), imipramine (0.1 mg/kg; tricyclic antidepressant), bupropion (1 mg/kg; dopamine and noradrenaline reuptake inhibitor), MK-801 (0.001 mg/kg; NMDA receptor antagonist), 7-nitroindazole (25 mg/kg; nNOS inhibitor), and TNF-α from mouse (0.001 fg/site). All these drugs were obtained from Sigma Chemical Co., St. Louis, USA, except bupropion that was obtained from Libbs Farmaceutica Ltda. (Brazil). α-Tocopherol was dissolved in mineral oil with 10% of ethanol and administered orally (p.o.); fluoxetine, imipramine, bupropion and MK-801 were dissolved in distilled water and administered by p.o. route; 7-nitroindazole was dissolved in saline with 5% Tween 80 and administered by intraperitoneal (i.p.) route; and TNF-α was dissolved in saline (0.9% NaCl) and administered by intracerebroventricular (i.c.v.) route. The drugs were freshly prepared before administration and administered in a volume of 10 ml/kg body weight (p.o. and i.p. route) or 5 μl/site (i.c.v.). Control animals received the appropriate vehicles.

I.c.v. administration was performed as described by Kaster et al. (2012). For injection, 26G needle was attached to a polypropylene cannula coupled to a 50 μl-Hamilton microsyringe. The mice were lightly anesthetized with ether (only for the loss of the postural reflex) and the administration was performed by inserting the needle

perpendicularly through the skull and no more than 2 mm into the brain of the mice. 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 midpoint on a line drawn through to the anterior base of the ears. The injections were performed by an experienced person, and after dissection of the brain of the animal, the success of the injection was examined, macroscopically, discarding animals whose injection has been held in place inappropriate or has caused cerebral hemorrhage (<5%).

To investigate the effect of α-tocopherol in an animal model of depressive-like behavior induced by TNF-α, mice were treated (p.o.) with vehicle or three doses of α-tocopherol (10, 30 or 100 mg/kg). After 30 min, they received an injection (i.c.v.) of TNF-α (0.001 fg/site, i.c.v.) or vehicle (saline). After another 30 min, the animals were subjected to behavioral tests. The dose of TNF-α and α-tocopherol was chosen based on previous studies from our group (Kaster et al., 2012; Lobato et al., 2010).

In another set of experiments, mice received (p.o.) vehicle or subeffective doses of fluoxetine, imipramine, bupropion or MK-801. Immediately after, they were treated with a subeffective dose of α-tocopherol (10 mg/kg, p.o.) or vehicle. After 30 min, they received an i.c.v. injection of TNF-α or vehicle, 30 min before being tested in the tail suspension test (TST) or open-field paradigm.

To investigate a possible synergistic effect between α-tocopherol and 7-nitroindazole, mice received (p.o.) vehicle or a subeffective dose of α-tocopherol. After 30 min, they received (i.p.) a subeffective dose of 7-nitroindazole or vehicle and immediately after, an injection (i.c.v.) of TNF-α or vehicle. The behavioral tests were performed 30 min after the end of the last treatment.

The doses of antidepressants, MK-801 and 7-nitroindazole used were chosen based on experiments previously performed in our laboratory (Binfaré et al., 2009; Brocardo et al., 2008).

2.3. Behavioral tests

2.3.1. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). 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. Mice were considered immobile only when they hung passively and completely motionless. The immobility time was recorded during a 6-min period by an experienced observer blind to the animal condition.

2.3.2. Open-field test

In order to rule out the possibility that the alteration in the immobility time in the TST was due to interference of the locomotor activity, the animals were subjected to an open-field test for 6 min, as described by Moretti et al. (2013). This test was carried out in a wooden box measuring 40 × 60 × 50 cm, with the floor of the arena divided into 12 equal squares. The number of squares crossed with all four paws (crossings) was manually counted and was the parameter used to evaluate locomotor activity. The apparatus was cleaned with a solution of 10% ethanol between tests in order to hide animal clues.

2.4. Biochemical analysis

An independent group of mice was used for biochemical analysis. Animals received (p.o.) vehicle, an active dose of bupropion (10 mg/kg) or two doses of α-tocopherol: 10 mg/kg (subeffective dose) or 30 mg/kg (active dose). After 30 min, the animals received an injection (i.c.v.) of TNF-α or vehicle (saline). After another 30 min, the animals were decapitated for biochemical analysis.

2.4.1. Tissue preparation

After decapitation, the brain of each animal was removed and the hippocampi were quickly dissected (4 °C), placed in liquid nitrogen and stored at −80 °C until use. The samples were prepared as described previously (Oliveira et al., 2008). Briefly, samples were mechanically homogenized in 400 μl of TRIS 50 mM pH 7.0, EDTA 1 mM, NaF 100 mM, PMSF 0.1 mM, Na_3VO_4 2 mM, Triton X-100 1%, glycerol 10%, and Sigma Protease Inhibitor Cocktail (P2714) and then incubated for 10 min in ice. Lysates were centrifuged (10.000 ×*g* for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in TRIS 100 mM pH 6.8, EDTA 4 mM, and SDS 8% and boiled for 5 min. Thereafter, sample dilution (40% glycerol, 100 mM TRIS, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β mercaptoethanol (final concentration 8%) were added on the samples. Protein content was estimated at 620 nm wavelength and the concentration calculated using a standard curve with bovine serum albumin as standard (Peterson, 1977).

2.4.2. Western blotting

The same amount of protein (60 μg per lane) for each sample was electrophoresed in SDS-PAGE minigels (10% acrylamide for the analysis of GSK-3β and 12% acrylamide for the analysis of Bax, Bcl2, and β-actin) and transferred to nitrocellulose membranes using a tank transfer system at 100 V and 270 mA for 1 h (Mini-PROTEAN Tetra Cell Electrophoresis System, Bio-Rad, Hercules, CA) (Cordova et al., 2004). To verify transfer efficiency process, gels were stained with Coomassie blue and membranes with Ponceau S.

The membranes were blocked with 5% skim milk in TBS (TRIS 10 mM, NaCl 150 mM, pH 7.5). GSK-3β total and phosphorylated (Ser9) forms, Bax and Bcl2 immunocontents were detected using specific antibodies incubated overnight diluted in TBS-T (10 mM TRIS, 150 mM NaCl, 0.1% Tween-20, pH 7.5) containing 2.5% BSA in the dilutions 1:1.000. Next, the membranes were incubated with anti-rabbit peroxidase-linked secondary antibody (1:5.000) for 1 h and the reactions developed by chemiluminescence (LumiGLOH, Cell Signaling, Beverly, MA, USA). All blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T. The optical density (O.D.) of the bands was quantified using the Scion Image™ (Frederick, MD, USA). The phosphorylation levels of GSK-3β were determined as a ratio of the O.D. of the phosphorylated band over the O.D. of the total band. The immunocontent of Bcl2 and Bax was determined as a ratio of the O.D. of the Bcl2 or Bax band over the O.D. of the β-actin band. Data were expressed as percentage of the control (considered as 100%).

The antibodies anti-phospho-GSK-3β (Ser9), anti-GSK-3β, anti-Bax, anti-Bcl2 and anti-β-actin detected a single band of approximately 46 kDa, 46 kDa, 20 kDa, 28 kDa and 45 kDa, respectively.

2.5. Statistical analysis

All data are presented as mean + SEM. Differences among experimental groups were determined by two-way or three-way ANOVA followed by Duncan's multiple range post hoc test, when appropriate. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Behavioral observations

3.1.1. Investigation of the acute effect of α-tocopherol in TNF-α-treated mice

Fig. 1A shows that systemic acute administration (p.o.) of α-tocopherol at doses of 30 and 100 mg/kg, produced a reduction in immobility time in the TST as compared to control mice, while the dose of 10 mg/kg was ineffective. Furthermore, the administration of TNF-α (i.c.v.) increased the immobility time in the TST, an effect prevented by the acute administration of the α-tocopherol at doses of

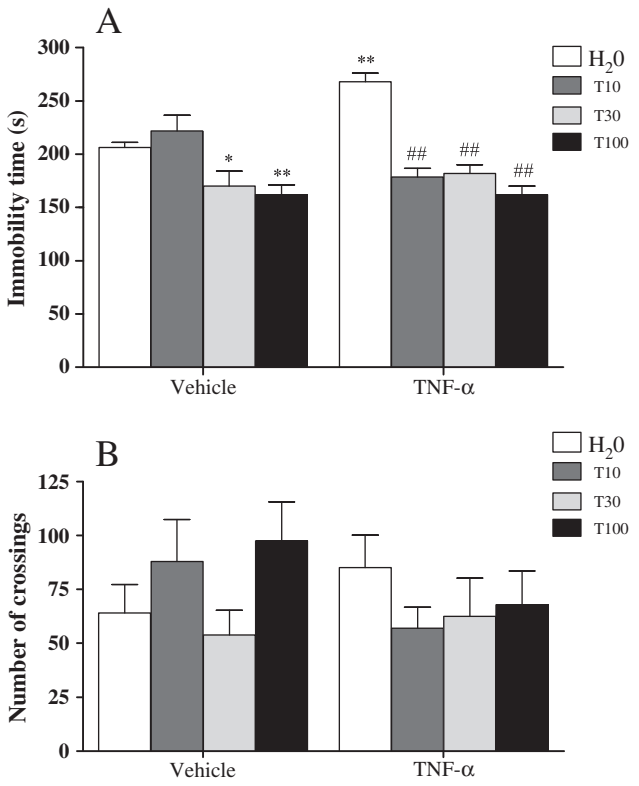

Fig. 1. Effect of treatment with α-tocopherol (T) at different doses (ranging from 10 to 100 mg/kg, p.o.) associated with administration of TFN-α (0.001 fg/site; i.c.v.) or vehicle on the immobility time in the TST (panel A) and on number of crossings in the open-field test (panel B). Bars represent means + SEM (n = 8). *$P < 0.05$ compared with the control group (vehicle). **$P < 0.01$ compared with control group (vehicle). ##$P < 0.01$ compared with the group treated with TNF-α. (Two-way ANOVA followed by Duncan's post hoc test).

10, 30 and 100 mg/kg ($P < 0.01$) (TNF-α treatment [$F(1,56) = 1.15$, $P > 0.05$], α-tocopherol treatment [$F(3,56) = 22.18$, $P < 0.01$], TNF-α × α-tocopherol [$F(3,56) = 9.57$, $P < 0.01$]).

None of the treatments caused changes in locomotor activity in the open-field test (Fig. 1B) (TNF-α treatment [$F(1,56) = 0.50$, $P > 0.05$], α-tocopherol treatment [$F(3,56) = 0.88$, $P > 0.05$], TNF-α × α-tocopherol treatment interaction [$F(3,56) = 1.48$, $P > 0.05$]).

3.1.2. Effects of the combined administration of α-tocopherol and antidepressants in TNF-α-treated mice

Fig. 2 shows that acute administration of a subeffective dose of α-tocopherol (10 mg/kg, p.o.) in combination with subeffective doses of fluoxetine (panel A), imipramine (panel C) or bupropion (panel E) decreased the immobility time in the TST, whereas the administration of drugs alone did not change the immobility time. Fig. 2 also shows that the administration of TNF-α produced an increased immobility time in the TST, which was prevented by the acute administration of α-tocopherol and antidepressants alone or in combination. In addition, in animals that received TNF-α, the combined treatment with α-tocopherol and imipramine (panel C) or bupropion (panel E) produced a synergistic antidepressant-like effect, since the immobility time in the TST was statistically lower as compared to the one obtained in the groups that received α-tocopherol, imipramine or bupropion alone (fluoxetine: TNF-α treatment [$F(1,54) = 0.50$, $P > 0.05$], α-tocopherol treatment [$F(1,54) = 28.60$, $P < 0.01$], fluoxetine treatment [$F(1,54) = 46.32$, $P < 0.01$], TNF-α × α-tocopherol treatment interaction [$F(1,54) = 10.45$, $P < 0.01$], TNF-α × fluoxetine treatment interaction [$F(1,54) = 2.12$, $P > 0.05$], α-tocopherol × fluoxetine treatment interaction [$F(1,54) = 1.29$, $P > 0.05$], TNF-α × α-tocopherol × fluoxetine treatment interaction [$F(1,54) = 15.56$, $P < 0.01$]; imipramine: TNF-α treatment [$F(1,56) = 0.03$, $P > 0.05$], α-tocopherol treatment [$F(1,56) = 19.65$, $P < 0.01$], imipramine treatment [$F(1,56) = 48.26$, $P < 0.01$], TNF-α × α-tocopherol treatment interaction [$F(1,56) = 17.78$, $P < 0.01$], TNF-α × imipramine treatment interaction [$F(1,56) = 5.66$, $P < 0.05$], α-tocopherol × imipramine treatment interaction [$F(1,56) = 0.162$, $P > 0.05$], TNF-α × α-tocopherol × imipramine treatment interaction [$F(1,56) = 4.98$, $P < 0.05$]; bupropion: TNF-α treatment [$F(1,56) = 0.38$, $P > 0.05$], α-tocopherol treatment [$F(1,56) = 30.54$, $P < 0.01$], bupropion treatment [$F(1,56) = 56.58$, $P < 0.01$], TNF-α × α-tocopherol treatment interaction [$F(1,56) = 11.52$, $P < 0.01$], TNF-α × bupropion treatment interaction [$F(1,56) = 1.34$, $P < 0.05$], α-tocopherol × bupropion treatment interaction [$F(1,56) = 0.01$, $P > 0.05$], TNF-α × α-tocopherol × bupropion treatment interaction [$F(1,56) = 18.10$, $P < 0.01$]).

None of the treatments caused changes in locomotor activity in the open-field test (Fig. 2B, D, F) (fluoxetine: TNF-α treatment [$F(1,56) = 0.02$, $P = 0 > 0.05$], α-tocopherol treatment [$F(1,56) = 0.80$, $P > 0.05$], fluoxetine treatment [$F(1,56) = 5.63$, $P < 0.05$], TNF-α × α-tocopherol treatment interaction [$F(1,56) = 0.06$, $P > 0.05$], TNF-α × fluoxetine treatment interaction [$F(1,56) = 0.41$, $P > 0.05$], α-tocopherol × fluoxetine treatment interaction [$F(1,56) = 0.53$, $P > 0.05$], TNF-α × α-tocopherol × fluoxetine treatment interaction [$F(1,56) = 0.03$, $P > 0.05$]; imipramine: TNF-α treatment [$F(1,54) = 0.67$, $P > 0.05$], α-tocopherol treatment [$F(1,54) = 3.84$, $P > 0.05$], imipramine treatment [$F(1,54) = 0.98$, $P > 0.05$], TNF-α × α-tocopherol treatment interaction [$F(1,54) = 0.30$, $P > 0.05$], TNF-α × imipramine treatment interaction [$F(1,54) = 0.06$, $P > 0.05$], α-tocopherol × imipramine treatment interaction [$F(1,54) = 1.70$, $P > 0.05$], TNF-α ×α-tocopherol × imipramine treatment interaction [$F(1,54) = 0.20$, $P > 0.05$]; bupropion: TNF-α treatment [$F(1,56) = 0.02$, $P > 0.05$], α-tocopherol treatment [$F(1,56) = 1.03$, $P > 0.05$], bupropion treatment [$F(1,56) = 3.12$, $P > 0.05$], TNF-α × α-tocopherol treatment interaction [$F(1,56) = 1.85$, $P > 0.05$], TNF-α × bupropion treatment interaction [$F(1,56) = 0.11$, $P > 0.05$], α-tocopherol × bupropion treatment interaction [$F(1,56) = 0.07$, $P > 0.05$], TNF-α × α-tocopherol × bupropion treatment interaction [$F(1,56) = 1.03$, $P > 0.05$]).

3.1.3. Effects of the combined administration of α-tocopherol and MK-801 or 7-nitroindazole in TNF-α-treated mice

Fig. 3 shows that acute administration of a subeffective dose of α-tocopherol in combination with subeffective doses of MK-801 (panel A) or 7-nitroindazole (panel C) reduced the immobility time in the TST in control mice (not treated with TNF-α), while the administration of these drugs alone did not change this parameter. The animals that received TNF-α had an increased immobility time in the TST. However, when mice that received TNF-α were pretreated with α-tocopherol, MK-801, 7-nitroindazole or the combined treatment with α-tocopherol and MK-801, no depressive-like behavior was observed. Interestingly, combined treatment with α-tocopherol and 7-nitroindazole (panel C) elicited a synergistic antidepressant-like effect in mice treated with TNF-α, since statistically lower immobility time values were obtained as compared to the values obtained in the groups of mice that received α-tocopherol or 7-nitroindazole alone (MK-801: TNF-α treatment [$F(1,56) = 12.73$, $P < 0.01$], α-tocopherol treatment [$F(1,56) = 45.69$, $P < 0.01$], MK-801 treatment [$F(1,56) = 33.89$, $P < 0.01$], TNF-α × α-tocopherol treatment interaction [$F(1,56) = 3.35$, $P > 0.05$], TNF-α × MK-801 treatment interaction [$F(1,56) = 0.25$, $P > 0.05$], α-tocopherol × MK-801 treatment interaction [$F(1,56) = 0.07$, $P > 0.05$], TNF-α × α-tocopherol × MK-801 treatment interaction [$F(1,56) = 41.91$, $P < 0.01$]; 7-nitroindale: TNF-α treatment [$F(1,54) = 4.74$, $P < 0.05$], α-tocopherol treatment [$F(1,54) = 30.06$, $P < 0.01$], 7-nitroindazole treatment [$F(1,54) = 62.95$, $P < 0.01$], TNF-α × α-tocopherol treatment interaction [$F(1,54) = 0.23$, $P > 0.05$], TNF-α × 7-nitroindazole treatment interaction [$F(1,54) = 2.58$, $P > 0.05$], α-tocopherol × 7-nitroindazole treatment interaction [$F(1,54) = 9.34$, $P < 0.01$], TNF-α ×α-tocopherol × 7-nitroindazole treatment interaction [$F(1,54) = 10.12$, $P < 0.01$]).

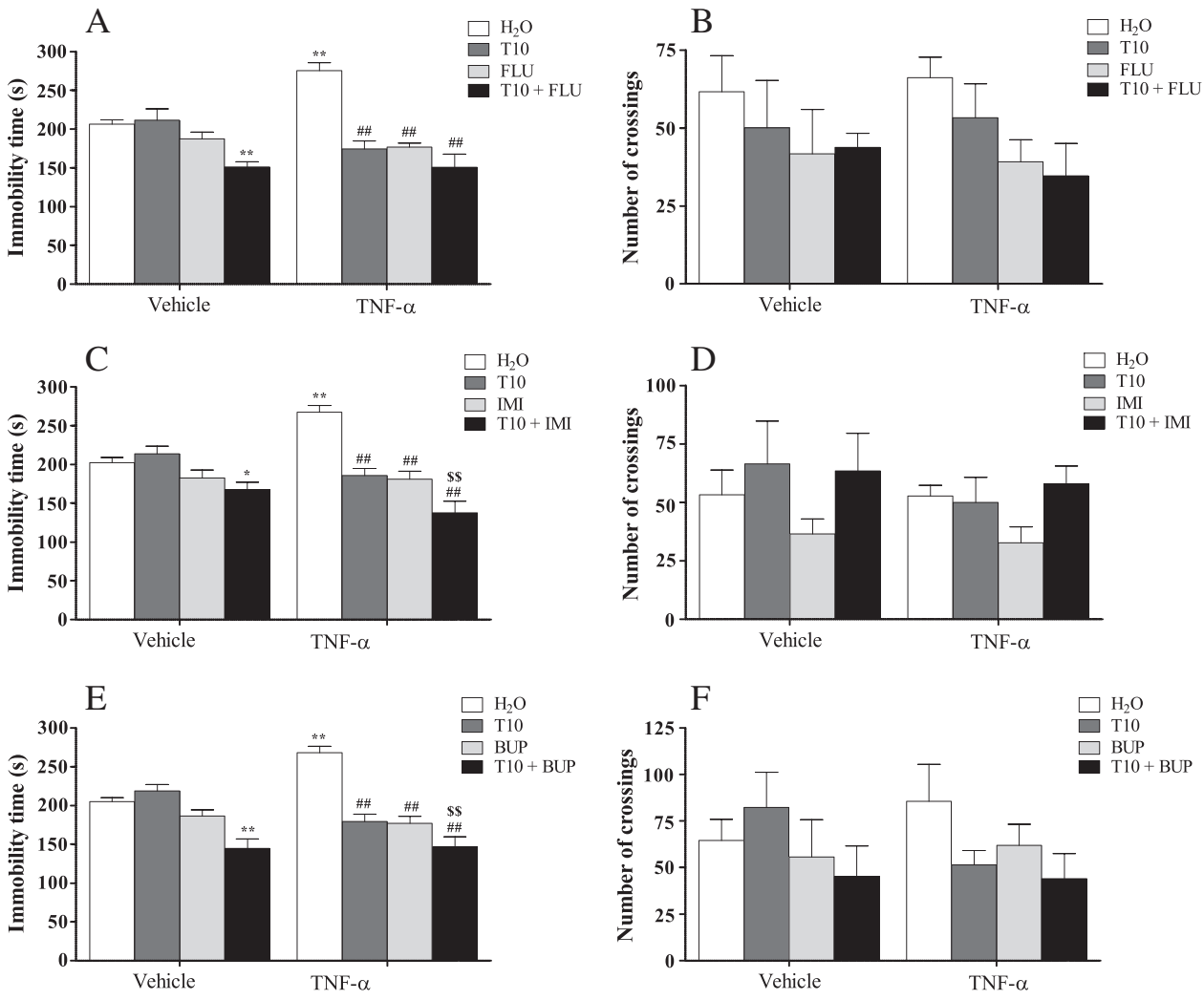

Fig. 2. Effect of treatment with a subeffective dose of α-tocopherol (T) (10 mg/kg, p.o.) and/or subeffective dose of fluoxetine (FLU) (5 mg/kg, p.o.), imipramine (0.1 mg/kg, p.o.) or bupropion (BUP) (1 mg/kg, p.o.) associated with administration of TFN-α (0.001 fg/site; i.c.v.) or vehicle on the immobility time in the TST (panels A, C, E) and on number of crossings in the open-field test (panels B, D, F). Bars represent means + SEM (n = 7–8). *$P < 0.05$ compared with the control group (vehicle). **$P < 0.01$ compared with control (vehicle). ##$P < 0.01$ compared with group treated with TNF-α. $$$P < 0.01$ compared with group T10/TNF-α and with the group antidepressant/TNF-α. (Three-way ANOVA followed by Duncan's post hoc test).

None of the treatments caused changes in locomotor activity in the open-field test (Fig. 3B, D) (MK-801: TNF-α treatment [$F(1,56) = 0.08$, $P > 0.05$], α-tocopherol treatment [$F(1,56) = 1.37$, $P > 0.05$], MK-801 treatment [$F(1,56) = 0.82$, $P > 0.05$], TNF-α × α-tocopherol treatment interaction [$F(1,56) = 0.50$, $P > 0.05$], TNF-α × MK-801 treatment interaction [$F(1,56) = 1.84$, $P > 0.05$], α-tocopherol × MK-801 treatment interaction [$F(1,56) = 0.02$, $P > 0.05$], TNF-α × α-tocopherol × MK-801 treatment interaction [$F(1,56) = 0.12$, $P > 0.05$]; 7-nitroindale: TNF-α treatment [$F(1,55) = 0.0005$, $P > 0.05$], α-tocopherol treatment [$F(1,55) = 0.02$, $P > 0.05$], 7-nitroindazole treatment [$F(1,55) = 6.80$, $P < 0.05$], TNF-α × α-tocopherol treatment interaction [$F(1,55) = 0.52$, $P > 0.05$], TNF-α × 7-nitroindazole treatment interaction [$F(1,55) = 0.09$, $P > 0.05$], α-tocopherol × 7-nitroindazole treatment interaction [$F(1,55) = 0.002$, $P > 0.05$], TNF-α × α-tocopherol ×7-nitroindazole treatment interaction [$F(1,55) = 0.04$, $P > 0.05$]).

3.2. Bax and Bcl2 immunocontent and GSK-3β phosphorylation

Fig. 4 shows a representative western blot of the hippocampal immunocontent of Bax (panel A) and Bcl2 (panel B) of mice injected with TNF-α and pretreated with α-tocopherol (10 and 30 mg/kg) or bupropion (10 mg/kg). Densitometric analysis revealed no changes in the content of Bax (panel A) and Bcl2 (panel B) in hippocampus of mice, independent on the treatment condition (Bax: TNF-α treatment [$F(1,24) = 0.2278$, $P > 0.05$], treatment [$F(3,24) = 0.0225$, $P > 0.05$], TNF-α ×treatment interaction [$F(3,24) = 0.3318$, $P > 0.05$]; Bcl2: TNF-α treatment [$F(1,24) = 1.4328$, $P > 0.05$], treatment [$F(3,24) = 0.2001$, $P > 0.05$], TNF-α × treatment interaction [$F(3,24) = 0.1221$, $P > 0.05$]). Similarly, no alteration in GSK-3β phosphorylation (Ser9) in the hippocampus was observed in any experimental group (panel C) (TNF-α treatment [$F(1,24) = 0.1178$, $P > 0.05$], treatment [$F(3,24) = 0.2869$, $P > 0.05$], TNF-α × treatment interaction [$F(3,24) = 0.3998$, $P > 0.05$]).

4. Discussion

This study demonstrated that: a) TNF-α administered acutely by i.c.v. route produces a depressive-like behavior in the TST; b) different doses of α-tocopherol, administered systemically (p.o.) and acutely were able to prevent the behavioral effects induced by TNF-α; c) the combined acute administration of subeffective doses of α-tocopherol with subeffective doses of antidepressants (fluoxetine, imipramine and bupropion), NMDA receptor antagonist (MK-801) and nNOS inhibitor (7-nitroindazole) caused a reduction of immobility time in the TST either in mice treated with TNF-α or in mice treated with vehicle; and d) treatment with TNF-α and/or α-tocopherol

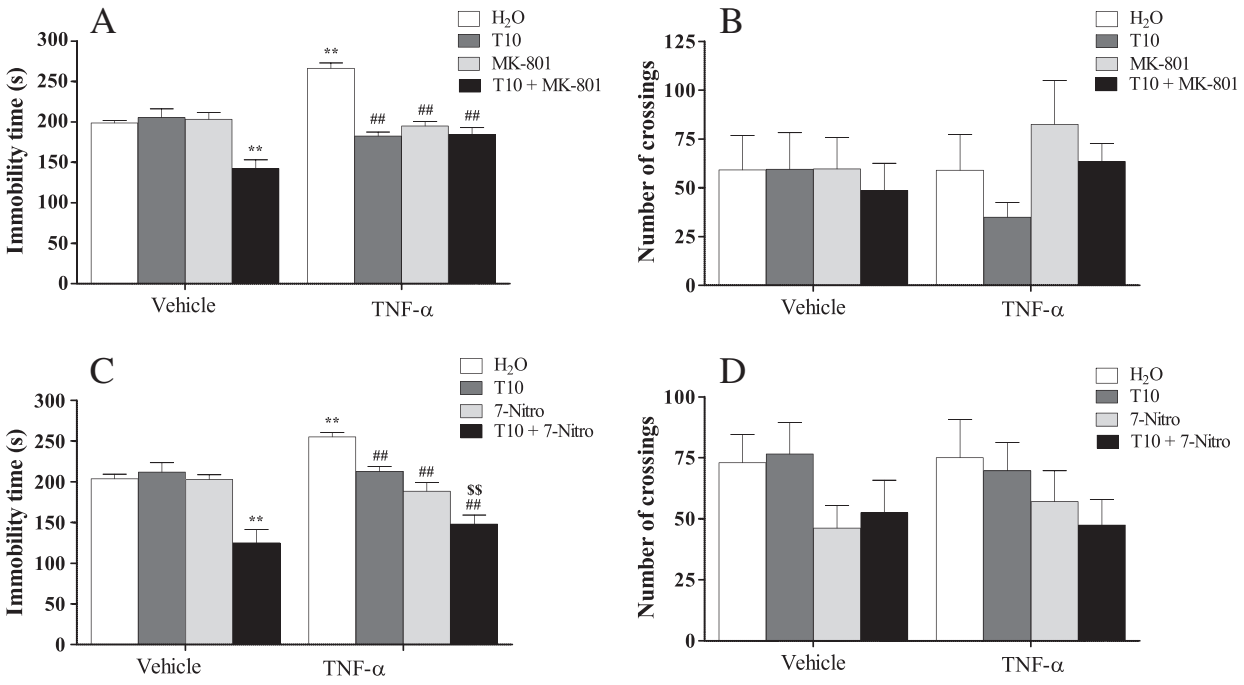

Fig. 3. Effect of treatment with subeffective dose of α-tocopherol (T) (10 mg/kg, p.o.) and/or subeffective dose of MK-801 (0.001 mg/kg, p.o.) or 7-nitroindazole (7-Nitro) (25 mg/kg, p.o.) associated with administration of TFN-α (0.001 fg/site; i.c.v.) or vehicle on the immobility time in the TST (panels A, C) and on number of crossings in the open-field test (panels B, D). Bars represent means + SEM (n = 7–8). $^{**}P < 0.01$ compared with control (vehicle). $^{\#\#}P < 0.01$ compared with group treated with TNF-α. $\$\$P < 0.01$ compared with group T10/TNF-α and with the group antidepressants/TNF-α. (Three-way ANOVA followed by Duncan's post hoc test).

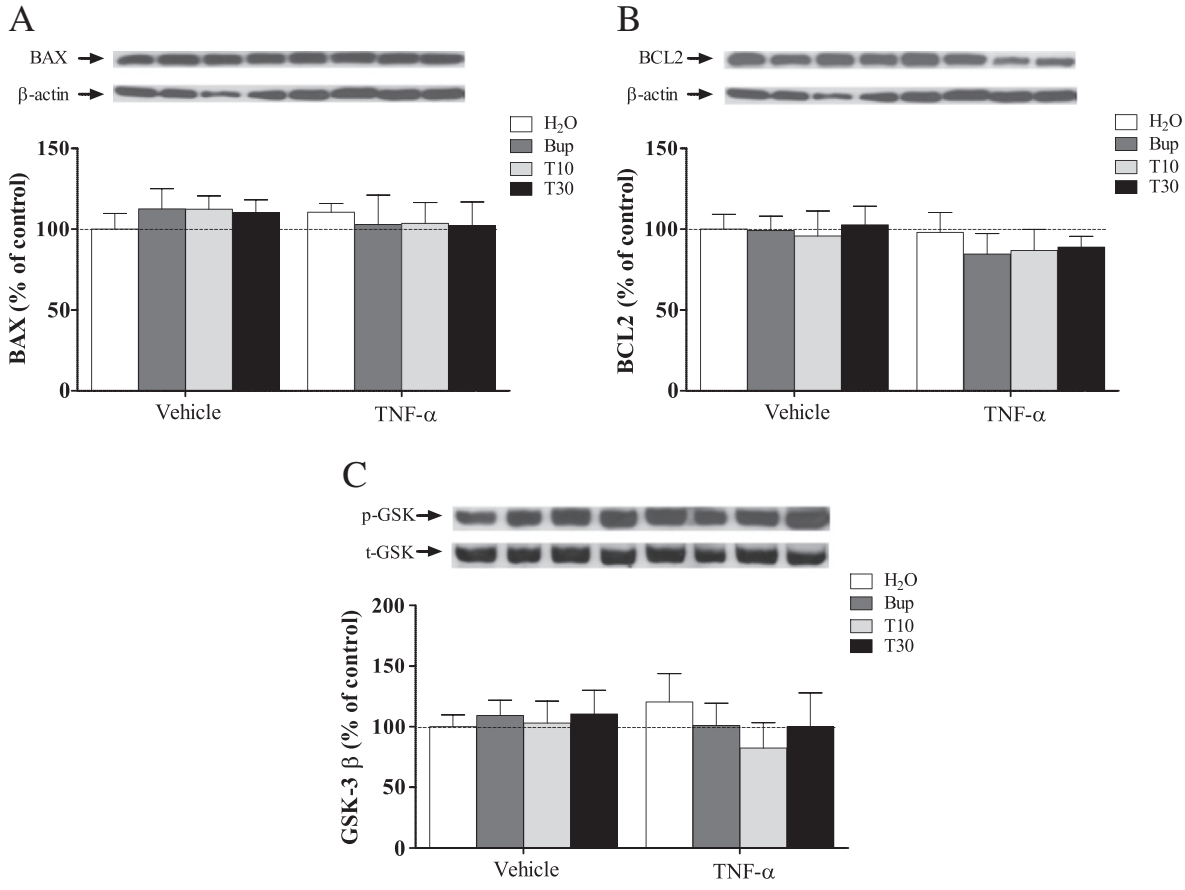

Fig. 4. Effect of treatment with α-tocopherol (T; 10–30 mg/kg, p.o.) or bupropion (BUP) (10 mg/kg, p.o.) associated with administration of TFN-α (0.001 fg/site; i.c.v.) or vehicle on the immunocontent of Bax (panel A), Bcl2 (panel B) and phosphorylation (Ser9) (panel C) of glycogen synthase kinase-3β (GSK-3β) in the hippocampus of mice. Immunocontent of Bax and Bcl2 and phosphorylation of GSK-3β were assessed by western blotting and revelation was performed by chemiluminescence. The densitometry of bands was determined using the Scion Image software®. A representative image and quantitative analysis normalized to the total-GSK-3β (t-GSK) (upper bands, p-GSK-3β; lower bands, t-GSK-3β) and β-actin (upper bands, Bax or Bcl2; lower bands, β-actin) bands are shown. The results are expressed as the percentage of vehicle control levels and represent the mean + SEM (n = 4) (two-way ANOVA).

failed to alter Bax and Bcl2 immunocontent or GSK-3β (Ser9) phosphorylation.

TNF-α is a protein that can be produced by several cell types, including monocytes, lymphocytes, macrophages, neurons and glial cells in response to infection, injury, inflammation or other environmental changes (Baud and Karin, 2001; Tchelingerian et al., 1994). This cytokine exerts its actions by interacting with specific receptors for tumor necrosis factor types 1 and 2, leading to activation of signaling cascades that regulate pro- and antiapoptotic pathways, cell proliferation and inflammation (Baud and Karin, 2001). The first demonstrations that depressed patients had increased TNF-α levels, were shown by Lanquillon et al. (2000), Mikova et al. (2001) and Kagaya et al. (2001), and this association has been confirmed by other studies and meta-analysis (Dowlati et al., 2010; O'Brien et al., 2007). Furthermore, psychological stress, which is also correlated with depression, also causes an increase on inflammatory cytokines, including TNF-α (Glaser and Kiecolt-Glaser, 2005; Lalive et al., 2002) and mice with TNF-α receptor deficiency had alteration in anxiety-like behavioral and neuroendocrine stress responses (Gimsa et al., 2012).

Herein we observed that administration of TNF-α induced a depressive-like behavior in the mouse TST, confirming a previous study (Kaster et al., 2012), which showed that the administration of the same dose of TNF-α increased the immobility time of mice exposed to TST. In rats, chronic administration of recombinant rat TNF-α (via i.c.v.) also elicits depressive-like behavior (Reynolds et al., 2004). Furthermore, Kaster et al. (2012) demonstrated that the use of inhibitor of TNF-α synthesis prevented the increase in immobility time induced by this cytokine in the forced swimming test (FST), reinforcing the idea that the elevated levels of TNF-α in mice brain are related to the depressive-like behavior in these animals. Corroborating these data, studies reported that TNF-α receptor knockout mice exhibited a decrease in immobility time in the TST and FST (Kaster et al., 2012; Simen et al., 2006) and that mice lacking the gene for TNF-α demonstrated a shorter duration of immobility in the FST when compared to control animals (Yamada et al., 2000).

In the present study, the acute administration of α-tocopherol 30 min before the injection of TNF-α was able to prevent the increase of immobility time caused by TNF-α. In line with this result, α-tocopherol attenuated the sickness behavior induced by lipopolysaccharides (Berg et al., 2004; Godbout et al., 2005) as well as decreased the expression of nuclear factor-κB (NFκB), IL-6, IL-1β and TNF-α in the brain of mice that had received lipopolysaccharide (Godbout et al., 2005). In addition, two preclinical studies using predictive tests for evaluation of antidepressant activity (TST and FST), showed that α-tocopherol reduces the duration of immobility time in mice (Lobato et al., 2010) and rats (Parveen, 2011). It is worth mentioning that, in our study, no significant change in the number of crossings in the open-field test after administration of TNF-α or α-tocopherol was shown, indicating that the behavioral effects of the cytokine and α-tocopherol observed in the present study are not due to any change in the locomotor activity. Indeed, our results are consistent with the fact that several classes of antidepressants such as tricyclics (amitriptyline, desipramine, imipramine), MAO inhibitors (clorgyline, moclobemide, nialamide, pargyline, toloxatone) and atypical antidepressants (bupropion, citalopram, indalpine, mianserin, nomifensine, viloxazine) decrease the duration of immobility time in the TST (Steru et al., 1987). Even these drugs taking several weeks to obtain an improvement in the clinical setting, they show antidepressant effect in the TST when administered acutely.

Another interesting result found in the present work is that the associated acute administration of subeffective doses of antidepressants (fluoxetine, imipramine and bupropion) with a subeffective dose of α-tocopherol elicited an antidepressant-like behavior in mice that received vehicle. This result is in agreement with the reported synergistic antidepressant-like effect in the FST elicited by the combined administration of α-tocopherol and fluoxetine (Lobato et al., 2010). Furthermore, subeffective doses of α-tocopherol and antidepressants alone or in combination prevented the depressive-like behavior induced by TNF-α and that the combined treatment with imipramine or bupropion and α-tocopherol resulted in a synergistic antidepressant-like effect in animals receiving TNF-α, suggesting that the antidepressant-like effect of α-tocopherol involves, at least in part, the modulation of serotonergic, dopaminergic and noradrenergic systems. It is well established the role of monoamine neurotransmitters (serotonin, noradrenaline, and dopamine) in depression (Andrews et al., 2011; Elhwuegi, 2004), and the involvement of monoaminergic neurotransmission in antidepressant-like response in animal models of depression (Elhwuegi, 2004; Yamada et al., 2004). Additionally, vitamin E was reported to interfere with monoamine levels in the brain of rats (Adachi et al., 1999; Castano et al., 1992). Castano et al. (1992) showed that 15 days of vitamin E-deficient diet led to a decrease in serotonin levels and an increase in dopamine levels in the rat brains. In the study performed by Adachi et al. (1999), vitamin E-deficient diet for 24 weeks decreased the levels of serotonin, dopamine and the activity of tryptophan hydroxylase enzyme (involved in the formation of serotonin) in the brain stem of rats. Likewise, similar to the finding of our study, other vitamins that also have antidepressant-like activity in animal models, such as ascorbic acid and folic acid, also seem to interact with monoaminergic systems to produce their behavioral effects (Binfaré et al., 2009; Brocardo et al., 2008).

In addition, some studies have shown that antidepressants that alter the monoaminergic system (e.g., fluoxetine, bupropion, citalopram) have antioxidant and anti-inflammatory properties (Abdel-Salam et al., 2004; Behr et al., 2012; Sacre et al., 2010; Zafir and Banu, 2007; Zafir et al., 2009). Therefore, we suggest that the antidepressant-like effect of α-tocopherol might be due, at least in part, from its antioxidant and/or anti-inflammatory actions. Studies have demonstrated that dietary supplementation of antioxidants with α-tocopherol (alone or in combination with other antioxidants), prevented oxidative stress in the rat brain (dos Santos et al., 2011; Thakurta et al., 2012) and that α-tocopherol dietary supplementation prevented oxidative stress and neuroglial over-activation, acting as an antioxidant and reducing neuroinflammation (Betti et al., 2011). In humans, supplementation with vitamin E also decreases lipid peroxidation, increases antioxidant enzyme activity (Shinde et al., 2011; Zal et al., 2011) and decreases inflammation (Devaraj et al., 2007; Wu et al., 2007).

Noteworthy, an antidepressant-like effect in animal models has been reported to be elicited by the administration of some nutrients, phytochemicals, herbal or substances with antioxidant and/or anti-inflammatory properties, such as omega-3 fatty acids (Venna et al., 2009), curcumin (Kulkarni et al., 2008), green tea polyphenols (Zhu et al., 2012), *Ginkgo biloba* extract (Rojas et al., 2011), coenzyme Q10 (Maes et al., 2009), N-acetylcysteine (Ferreira et al., 2008), and ascorbic acid (Binfaré et al., 2009; Moretti et al., 2012).

The glutamatergic system and the L-arginine–NO pathway have also been reported to be associated with psychiatric disorders (Dhir and Kulkarni, 2011; Skolnick, 1999; Zarate et al., 2010). Several preclinical studies have reported that NMDA receptor antagonists exert antidepressant-like effects in animal models of depression (Dhir and Kulkarni, 2008; Li et al., 2011) and that compounds with antidepressant properties have their effect potentiated by the reduction of NO levels. In addition, an increased level of NO prevents the antidepressant-like activity of these compounds (Bettio et al., 2012; Brocardo et al., 2008; Moretti et al., 2011). Human studies have also shown that NMDA receptor antagonists are capable of decreasing depressive symptoms (DiazGranados et al., 2010; Zarate et al., 2006), and that depressed patients who attempted suicide had higher plasma levels of NO than the control ones (Kim et al., 2006).

Furthermore, excessive amounts of nitric oxide may react with superoxide anions, which generate peroxynitrite anions and peroxynitrous acid, causing tissue toxicity and stimulating nitrosative stress, factors that are associated with depressive symptoms (Maes et al., 2011).

Activated oxidative and nitrosative stress pathways may contribute to depression through multiple mechanisms, e.g. damage to DNA, mitochondria, proteins and membrane polyunsaturated fatty acid omega-3 contents; damage to intracellular signaling molecules involved in the pathophysiology of depression; superinduction of the enzyme that converts tryptophan to the kynurenine pathway (Leonard and Maes, 2012).

In the present study, the acute administration of subeffective doses of α-tocopherol, NMDA receptor antagonist or nNOS inhibitor alone or in combination abolished the depressive-like behavior induced by TNF-α. Moreover, the combination of α-tocopherol with 7-nitroindazole resulted in a synergistic antidepressant-like effect in animals treated with TNF-α, suggesting that a modulation of NO production may account for the beneficial effects of α-tocopherol in this experimental protocol.

It is worth mentioning that some studies also demonstrate that α-tocopherol may influence the glutamatergic system, since it was reported to prevent the glutamate-induced increase in intracellular calcium concentration and production of reactive oxygen species as well as DNA and mitochondrial damage in HT4 neuronal cells (Tirosh et al., 2000). In addition, α-tocopherol was effective in lowering intracellular calcium in rat brain synaptosomes treated with glutamate (Avrova et al., 1999). Regarding L-arginine–NO pathway, although some studies have linked the beneficial effects of α-tocopherol with higher activity of NOS and consequent increase in NO production (Heller et al., 2004; Li et al., 2001; Tronchini et al., 2010), these studies investigated only peripheral actions of vitamin E, not evaluating issues related to the CNS and nNOS, specifically. However, taking into account that α-tocopherol is an antioxidant, it can prevent/avoid some of the damage caused by oxidative and nitrosative stress (Leonard and Maes, 2012). Therefore, adequate levels of vitamin E would be interesting for patients with depression. Corroborating this information, studies have shown that depressive patients have lower levels of vitamin E (Maes et al., 2000; Owen et al., 2005); that decrease in the dietary intake of vitamin E was associated with depression (German et al., 2012); and that nurses with high job stress had more severe depressive symptoms and lower plasma concentrations of α-tocopherol (Tsuboi et al., 2006).

After obtaining the results discussed so far, we evaluated if the animal model of depressive-like behavior induced by TNF-α would lead to any changes in proteins involved in apoptotic pathways, as well as the effect of α-tocopherol in this experimental paradigm. Literature data have demonstrated that, in vitro, IFN-γ (an inflammatory cytokine) was able to increase the levels of Bax and decrease levels of Bcl2 (Ning et al., 2010) and that TNF-α was able to increase the nuclear expression of GSK-3β (Park et al., 2011). In addition to the well-known effect of inflammation in apoptotic pathways, a recent review correlated several animal models of depressive-like behavior (chronic mild stress, learned helplessness) with increased inflammation and alteration in apoptotic pathways, including decreased Bcl2 expression (Kubera et al., 2011). In this work, no significant alteration was observed in the levels of Bax and Bcl2 and phosphorylation of GSK-3β (Ser9) in the hippocampus of mice that received TNF-α. However, the Bcl2 results should be considered with caution, because although not statistically significant, a slight decrease on Bcl2 immunocontent in animals treated with bupropion or α-tocopherol associated with TNF-α was shown. We do not rule out the possibility that it could attain a statistical significance with an increase in the number of samples analyzed.

A recent study performed by our group showed that acute restraint stress elicited depressive-like behavior and increased oxidative stress in the brain of mice (Moretti et al., 2013), alterations that could be result in a neutrophil-mediated inflammation (Juurlink and Paterson, 1998). However, somewhat similar to our study, Moretti et al. (2013) observed no effect of stress on GSK-3β phosphorylation or on BAX levels, while only an increased hippocampal level of Bcl2 was reported.

Studies also demonstrated that chronic treatment with antidepressants (such as fluoxetine, citalopram, desipramine, imipramine) modifies the Bax and Bcl2 levels (Bachis et al., 2008; Huang et al., 2007; Murray and Hutson, 2007) and that acute treatment with fluoxetine or imipramine altered GSK-3 phosphorylation (Li et al., 2004; Roh et al., 2005; Su et al., 2012). However, citalopram, imipramine and amitriptyline, when given acutely, failed to alter the levels of Bcl2 (Murray and Hutson, 2007). Here, administration of α-tocopherol or bupropion in an active dose did not significantly affect the levels of Bax and Bcl2 or phosphorylation of GSK-3β. However, studies in the literature show that vitamin E can alter the levels of Bax and/or Bcl2 or GSK-3 phosphorylation in different cell types and experimental protocols (Magalhães et al., 2007; Marsh et al., 2005). Studies involving the CNS show that: a) in rat cortical neurons, the administration of α-tocopherol prevented cell death induced by hydrogen peroxide, besides increasing Bcl2 expression via mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) (Numakawa et al., 2006); b) in rats, treatment with α-tocopherol prevented the increase of the Bax:Bcl2 ratio in the hippocampus and caudate nucleus induced by haloperidol — a medication used for treating psychotic disorders (Post et al., 2002); and c) α-tocopherol was also able to inhibit dephosphorylation of GSK-3β in oligodendrocytes treated with 7-ketocholesterol (which induces apoptosis) (Ragot et al., 2011).

One drawback of the present study is that the evaluation of Bcl2 and BAX was performed in a time period that was likely not enough to cause alterations in the expression of proteins involved in the control of apoptosis. Also, particularly regarding Bcl2 immunocontent, the number of samples used may not have been enough to detect a significant statistical difference. Therefore, the possibility that TNF-α and/or α-tocopherol may modulate the apoptotic pathway mediated by these proteins cannot be ruled out. We believe that the differences between the results obtained in our study and those available in the literature may be also due to the duration of α-tocopherol treatment.

5. Conclusions

The administration of TNF-α elicited a depressive-like behavior in mice exposed to TST, which was prevented by pre-administration of α-tocopherol. Moreover, a synergistic antidepressant-like effect of α-tocopherol with antidepressants was shown, even in the presence of an inflammatory insult, suggesting that this vitamin may be interesting to optimize conventional pharmacotherapy for depression, including depressive states associated with inflammatory conditions. Hence, the present study suggests that α-tocopherol might improve the effectiveness of antidepressant compounds used in the clinical and that this vitamin may be useful in treating patients with intolerable side effects or who are resistant to therapy with a single antidepressant. However, additional preclinical studies and clinical trials are needed to better elucidate this effect.

Acknowledgments

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