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ARTICLE

Behavioral and neurochemical effects of alpha lipoic acid associated with omega-3 in tardive dyskinesia induced by chronic haloperidol in rats

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Abstract: Tardive dyskinesia (TD) is characterized by involuntary movements of the lower portion of the face being related to typical antipsychotic therapy. TD is associated with the oxidative imbalance in the basal ganglia. Lipoic acid (LA) and omega-3 (ω-3) are antioxidants acting as enzyme cofactors, regenerating antioxidant enzymes. This study aimed to investigate behavioral and neurochemical effects of supplementation with LA (100 mg/kg) and ω-3 (1 g/kg) in the treatment of TD induced by chronic use of haloperidol (HAL) (1 mg/kg) in rats. Wistar male rats were used, weighing between 180–200 g. The animals were treated chronically (31 days) with LA alone or associated with HAL or ω-3. Motor behavior was assessed by open-field test, the catalepsy test, and evaluation of orofacial dyskinesia. Oxidative stress was accessed by determination of lipid peroxidation and concentration of nitrite. LA and ω-3 alone or associated caused an improvement in motor performance by increasing locomotor activity in the open-field test and decreased the permanence time on the bar in the catalepsy test and decreased the orofacial dyskinesia. LA and ω-3 showed antioxidant effects, decreasing lipid peroxidation and nitrite levels. Thus, the use of LA associated with ω-3 reduced the extrapyramidal effects produced by chronic use of HAL.

Key words: lipoic acid, omega-3, tardive dyskinesia, haloperidol, oxidative stress.

Résumé : La dyskinésie tardive (DT) se caractérise par des mouvements involontaires du bas du visage liés à un traitement par des antipsychotiques typiques. La DT est associée à un déséquilibre oxydatif dans les ganglions de la base. L'acide lipoïque (AL) et l'oméga 3 (ω-3) sont des antioxydants qui agissent comme cofacteurs enzymatiques participant à la régénérescence des enzymes antioxydants. Ces travaux visaient à étudier les effets comportementaux et neurobiochimiques de la supplémentation en AL (100 mg/kg) et en ω-3 (1 g/kg) dans le traitement de la DT induite par l'administration chronique d'halopéridol (HAL) (1 mg/kg) chez le rat. Nous avons utilisé des rats Wistar mâles pesant de 180 à 200 g. Nous avons administré chroniquement (31 jours) aux animaux de l'AL seul ou en association avec de l'HAL ou de l'ω-3. Nous avons évalué le comportement moteur à l'aide du test en espace ouvert, du test de catalepsie et de l'évaluation de la dyskinésie orofaciale. Nous avons évalué le stress oxydatif en établissant le taux de peroxydation lipidique et les concentrations de nitrite. Seuls ou en association, l'AL et l'ω-3 permettaient d'améliorer les performances motrices en entraînant une augmentation de l'activité de locomotion dans le test en espace ouvert, une diminution du temps d'attente sur la barre dans le test de catalepsie et une diminution de la dyskinésie orofaciale. L'AL et l'ω-3 ont montré des effets antioxydants avec la diminution de la peroxydation lipidique et des taux de nitrite. Par conséquent, l'utilisation d'AL en association avec l'ω-3 permet de réduire les effets extrapyramidaux entraînés par l'administration chronique d'HAL. [Traduit par la Rédaction]

Mots-clés : acide lipoïque, oméga 3, dyskinésie tardive, halopéridol, stress oxydatif.

Introduction

Antipsychotics are a group of psychoactive drugs used to treat schizophrenia. The first generation drug described as typical antipsychotics, such as chlorpromazine, haloperidol (HAL), and sulpiride, present main mechanism of action blocking the dopamine D2 receptor. This blockage produces the desired therapeutic effects and side effects, the tardive dyskinesia (TD) and catalepsy main drawbacks of this class of drugs (Menegatti et al. 2004).

TD is characterized by repetitive, involuntary movements. The most serious aspect of TD is that it may persist for months or years after the drug withdrawal and in some patients are irreversible (Glazer et al. 1990). Thus, TD has been considered an important clinical problem in the treatment of schizophrenia.

The catalepsy is a typical side effect produced by chronic treatment with neuroleptics. It is characterized by loss of voluntary movement, impaired postural stability, inability to actively initi-

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ate movements, and persistent abnormal postures. This effect is due to the blockade of D2 receptors at the central level, especially in striatal areas. The catalepsy development occurs after 30 days of treatment with HAL and this effect correlates with reduced locomotor activity. These effects disappeared after 3 days of suspension of the drug. After 7 days of this suspension, up-regulation of D2 receptors in the striatum was observed. These phenomena are due to blockade of D2 receptors, although other mechanisms may also be involved (Vasconcelos et al. 2003). The catalepsy test is used for an important model for predicting extrapyramidal effects induced by neuroleptics (Chittiprol et al. 2010).

Evidence suggests that oxidative stress plays an important role in the pathophysiology of TD and catalepsy, a fact proved by studies published about this topic (Macêdo et al. 2011; de Oliveira et al. 2013). Some of these studies have reported problems with the antioxidant defense and increased lipid peroxidation in animals chronically treated with HAL (Aguiar et al. 2010; Lister et al. 2014; Macêdo et al. 2011; de Oliveira et al. 2013).

Based on the presence of oxidative stress in the pathophysiological process of TD, studies have been investigating the use of antioxidant therapies in an attempt to minimize extrapyramidal effects produced by chronic use of these typical antipsychotics (Daya et al. 2011; Macêdo et al. 2011; Peroza et al. 2013). Among these antioxidant therapies, we can mention the alpha lipoic acid (LA) (Thaakur and Himabindhu 2009) and omega-3 (ω-3) as potent antioxidants (Barcelos et al. 2010).

The LA is an antioxidant naturally synthesized in the human body and has been used to treat various diseases when provided as an oral supplement. Besides acting against free radicals, it promotes the reduction of lipid peroxidation, acts as a cofactor in many enzyme complexes, and regenerates damaged tissues (de Araújo et al. 2011; Ferreira et al. 2009). LA has the ability to combat reactive oxygen species (ROS) both in the lipophilic and hydrophilic environment (Packer et al. 1995). Due to its powerful antioxidant effect, LA would also be able to prevent neuronal damage caused by ROS produced during neurodegenerative diseases (de Araújo et al. 2013). In addition, dihydrolipoic acid, the reduced form of the LA, is able to regenerate other antioxidants of low molecular weight, such as glutathione, coenzyme Q10, and vitamins A and C (Packer et al. 1995). It is also attributed to this substance anti-inflammatory activity, and therefore the effect of short- and long-term reduction in oxidative processes related to neurodegenerative diseases. Furthermore, it works as a metal chelator, reducing ROS production (de Araújo et al. 2013; Silva et al. 2013).

The ω-3 is an essential polyunsaturated fatty acid (PUFA-n3) being acquired from external sources through diet or supplementation (Cardoso 2009).

The ω-3 shows a fundamental role in maintaining neuronal integrity to promote brain development and synaptic plasticity. Also, ω-3 present antioxidant effect by promoting the removal of ROS or interfere with the production of ROS (Barcelos et al. 2010). The EPA and DHA can alter production of catecholamines like dopamine and serotonin, are fundamental for the maintenance of motor function controlled by the dopaminergic system in the corpus striatum (Delattre et al. 2010).

Considering that oxidative stress is involved in the pathophysiology of dyskinesias induced by chronic use of HAL, and the ω-3 (Barcelos et al. 2010) and LA (de Araújo et al. 2013) have antioxidant effects proven in the literature, we hypothesized that the LA and (or) ω-3 co-administration could prevent the development of these dyskinesias. Therefore, the aim of this study was to evaluate the behavioral and neurochemical effects of LA and (or) ω-3 in HAL-induced TD in rodents.

Materials and methods

Animals

Male Wistar rats (weighing 180–200 g) were used, 8 per group from the animal colony of UERN. The animals were housed at a temperature of 24 ± 2 °C in a 12 h light – 12 h dark cycle, receiving food and water ad libitum. The study was approved by the Ethics Committee on Animal Research UERN under the protocol 001/12.

Drugs

Lipoic acid and HAL were purchased from Sigma–Aldrich (USA). ω-3 fish oil EPA-DHA 180/120 mg (1000 mg) were purchased from TopTherm (Brazil).

Experimental protocol

The animals were treated with saline (control), LA (100 mg/kg) by gavage, or HAL (1 mg/kg) intraperitoneally, or ω-3 fish oil EPA-DHA 180/120 mg (1 g/kg) by gavage alone or with associations of the (LA + ω-3), or (ω-3 + LA + HAL), or (LA + HAL), or (ω-3 + HAL). The drugs were administered chronically (31 days).

The open-field tests and catalepsy were performed after 31 days. The orofacial dyskinesia test was performed on 11, 21, and 31 days of treatment. All behavioral tests were performed 1 h after drug administration. After behavioral tests, the animals were euthanized by guillotine, the brain of the animals were removed, and the brain areas of interest (HC, hippocampus; PFC, prefrontal cortex; S, striatum) dissected on ice and stored at –70 °C to perform the neurochemical tests (Supplementary Fig. S1[1]).

Analysis of motor behavior

Open-field test

The open-field test area was made of timber (50 cm × 50 cm × 50 cm) divided into 4 squares of equal area. The open-field test was used to evaluate the exploratory activity of the rats. Each rat was placed in the center of the arena and the number of squares crossed, with the 4 paws (locomotor activity) was recorded for 5 min after 1 min of habituation. Before introducing each animal, the arena was cleaned with 10% alcohol to eliminate the possible bias due to the odor that could be left by previous animals (Archer 1973).

Catalepsy test

In this test, front legs of the animals were placed on a rigid bar 2 cm thick and 15 cm in height. Each rat was placed with its forepaws near the edge of the bar and the amount of time spent in this atypical position was recorded for 3 times: 60 min, 90 min, and 120 min after drug administration. All the rats treated were individually placed on the inclined grid and observed for the 60 s (Sanberg et al. 1988).

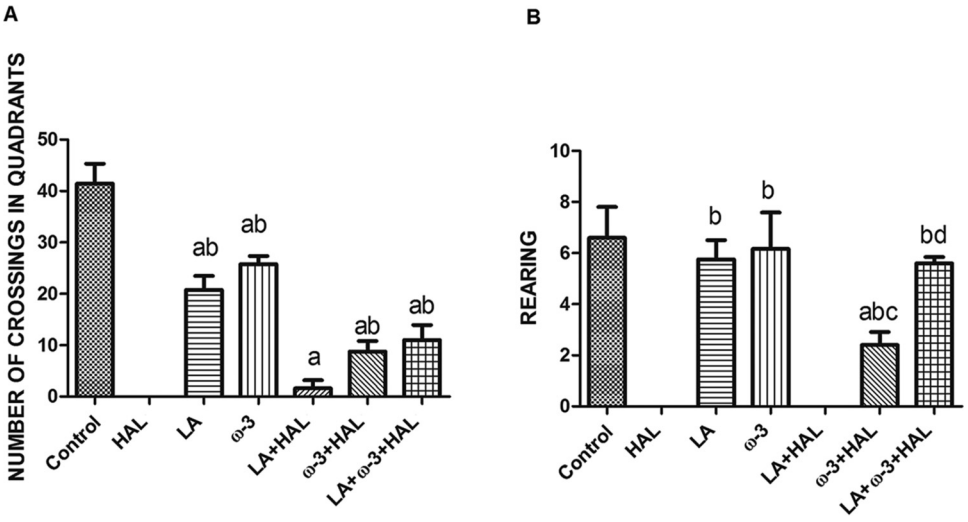
Evaluation of orofacial dyskinesia

The animals were placed on a scale similar to the open-field test arena, with mirrors at the base and sides. Thus, the observer had higher viewing angles. The animals were individually assessed by evaluating the number of vacuous chewing movement (VCM) and protrusions of the tongue (PT).

In this study, VCM is referred to as openings in the vertical plane, not facing physical material. The PT is referred to as the stereotypical behavior of the tongue with protrusions. If the PT or VCM occurs over a period of preparation, they will not be taken into account. The counting is stopped whenever the animal starts grooming. The VCM and PT were measured continuously for 6 min after a period of adaptation (6 min) (Naidu et al. 2003).

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjpp-2016-0307.

Fig. 1. Determination of the effect of lipoic acid (LA) and (or) omega-3 (ω-3) alone or associated with haloperidol (HAL) on the number of crossings in the quadrants (A) and rearing (B) in the open-field test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey's test as post hoc test were used. Lowercase letters above bars: a vs. the control, b vs. HAL, c vs. ω-3, d vs. ω-3 + HAL with $p < 0.0001$.



Neurochemical study

Evaluation of lipid peroxidation

Brain areas, the PFC, HC, and S, from all groups were dissected to prepare 10% homogenates (*w*/*v*, in 1.15% KCl). The formation of lipid peroxides during lipid peroxidation was followed by measuring the thiobarbituric acid reactive substances (TBARS), as previously described by Draper and Hadley. Briefly, samples were mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The reaction media was heated in a boiling water bath for 15 min, and *n*-butanol (2:1 *v*/*v*) was added to the media. After centrifugation (800*g*, 5 min), TBARS contents were determined at 535 nm. The results were expressed as micromoles of malondialdehyde (MDA) per mg protein (Draper and Hadley 1990).

Nitrite determination

Tissue samples from PFC, HC, or S were used to prepare 10% homogenates (*w*/*v*). After centrifugation (800*g*, 10 min), supernatants were collected and the nitric oxide (NO) production was determined by the Griess reaction. Briefly, 100 μL of the supernatant were incubated with 100 μL of the Griess reagent (1% sulfanilamide in 1% H_3PO_4 – 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride – 1% H_3PO_4 – distilled water (1:1:1:1)) at room temperature for 10 min. The absorbance was measured at 550 nm microplate reader. Nitrite concentration (μmol/L) was determined from a standard $NaNO_2$ curve (Green et al. 1981).

Statistical analysis

All tests were analyzed by one-way ANOVA using Prism 5.0 software. For meaningful results, multiple comparisons were made by the Tukey as the post hoc tests. Results were considered significant at $p < 0.05$ and presented as mean ± SEM.

Results

Behavioral tests

Open-field test

In the open-field test, HAL group (0.0 ± 0.0) showed a significant decrease in locomotor activity compared with the control group (41.4 ± 3.9) ($p < 0.0001$). In LA group alone (20.7 ± 2.7) as well as in the ω-3 group alone (25.7 ± 1.6), a decrease in the number of intersections of the quadrants was observed when compared with the control group and increased when compared with the HAL group ($p < 0.0001$) (Fig. 1A).

In the groups of associations with LA and HAL (HAL + LA: 1.6 ± 1.6) or ω-3 and HAL (ω-3 + HAL: 8.7 ± 2.0) or LA, ω-3, and HAL group (ω-3 + LA + HAL: 11.0 ± 2.9), all showed reduced locomotor activity when compared with control group. However, the latter 2 groups showed an increase in locomotor activity when compared with the HAL group (Fig. 1A).

In the evaluation of vertical exploratory activity (rearing), the results showed that LA group (5.7 ± 0.7) or the ω-3 alone group (6.2 ± 1.4) increased the frequency of rearing compared with the HAL group (0.0 ± 0.0). The associations of ω-3 and HAL (2.4 ± 0.5) or LA, ω-3, and HAL group (5.6 ± 0.2) ($p < 0.009$) showed increasing the number of rearing compared with the HAL group (Fig. 1B).

Catalepsy test

In the catalepsy test, the HAL group (60 min: 150.3 ± 29.3; 90 min: 186.8 ± 25.5; 120 min: 186.4 ± 26.0) remained longer on the bar than did the control group (60 min: 0.5 ± 0.5; 90 min: 4.1 ± 4.1; 120 min: 5.8 ± 5.8). The groups treated with LA alone (60 min: 0.6 ± 0.6; 90 min: 3.0 ± 3.0; 12 min: 5.8 ± 5.8) or ω-3 alone (60 min: 0.6 ± 0.6; 90 min: 4.0 ± 4.0; 120 min: 6.0 ± 6.0) or associations, HAL and LA (60 min: 93.0 ± 19.0; 90 min: 76.2 ± 9.0; 120 min: 71.6 ± 7.1) or ω-3 and HAL (60 min: 8.6 ± 4.1; 90 min: 43.4 ± 23.9; 120 min: 50.0 ± 15.3) or LA, ω-3 and HAL (60 min: 1.6 ± 1.4; 90 min: 5.1 ± 2.4; 120 min: 22.3 ± 12.8) were able to decrease the time spent on the bar when compared with the HAL group (Fig. 2).

Evaluation of orofacial dyskinesia

In the orofacial dyskinesia test, the HAL group (11th day: 13 ± 1.8; 21st day: 17.1 ± 3.3; 31st day: 10.3 ± 1.3) showed an increase in the VCM compared with the control group (11th day: 0.2 ± 0.2; 21st day: 0.2 ± 0.2; 31st day: 0.2 ± 0.2). The LA group (11th day: 0.2 ± 0.2; 21st day: 0.2 ± 0.2; 31st day: 0.2 ± 0.2) or ω-3 group (11th day: 0.2 ± 0.2; 21st day: 0.4 ± 0.2; 31st day: 2.4 ± 1.5) or the group treated with LA and HAL (11th day: 2.6 ± 0.4; 21st day: 4.0 ± 0.9; 31st day: 3.0 ± 0.7) were able to decrease the number of VCM when compared with the HAL group ($p < 0.0001$) (Fig. 3).

The association group ω-3 and HAL on the 11th day (5.2 ± 1.4) showed an increase in the number of VCM compared with the control group (0.2 ± 0.2) or ω-3 alone group (0.2 ± 0.2). Also in relation to the association group with ω-3 and HAL, this decreased the number of ECM compared with the HAL group in the 3 time periods analyzed. The association group with LA, ω-3, and HAL (11th day: 4.5 ± 1.0; 21st day: 3.9 ± 1.0; 31st day: 3.5 ± 0.8) exhibited

Fig. 2. Determination of the effect of lipoic acid (LA) and (or) omega-3 (ω-3) alone or associated with haloperidol (HAL) in time spent on the bar in the catalepsy test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey's test as post hoc test were used. Lowercase letters above bars: a vs. control, b vs. HAL, c vs. ω-3, with $p < 0.0001$.

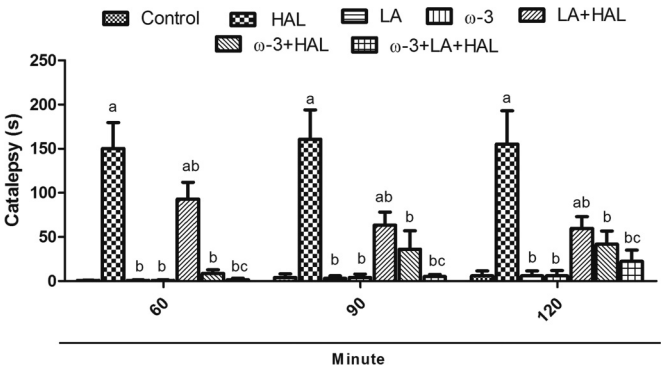
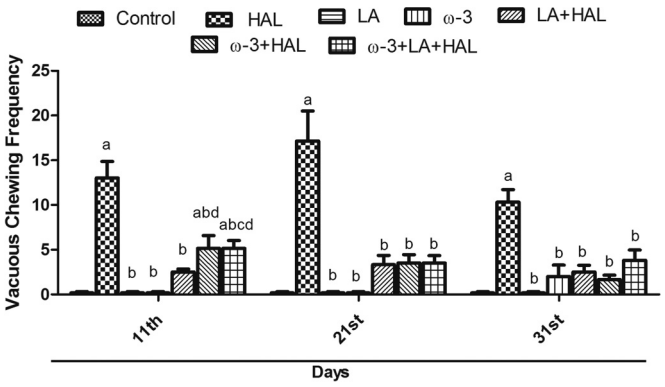


Fig. 3. Determination of the effect of lipoic acid (LA) and (or) omega-3 (ω-3) alone or associated with haloperidol (HAL) on the number of chewing motions in vacuo at orofacil dyskinesia test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey's test as post hoc test were used. Lowercase letters above bars: a vs. control, b vs. HAL, c vs. LA, d vs. ω-3 with $p < 0.0001$.



on the 11th day, increase in VCM compared with the control group (0.2 ± 0.2) or the LA group (0.2 ± 0.2) alone. However, when compared with the HAL group with association LA, ω-3 and HAL decreased the number of VCM in the 3 time periods studied ($p < 0.0001$) (Fig. 3).

Also, in the orofacial dyskinesia test, the HAL group (11th day: 5.6 ± 1.1; 21st day: 4.5 ± 0.8; 31st day: 5.2 ± 1.2) showed an increase in the number of PT when compared with the control group (11th day: 0.0 ± 0.0; 21st day: 0.0 ± 0.0; 31st day: 0.0 ± 0.0) (Fig. 4).

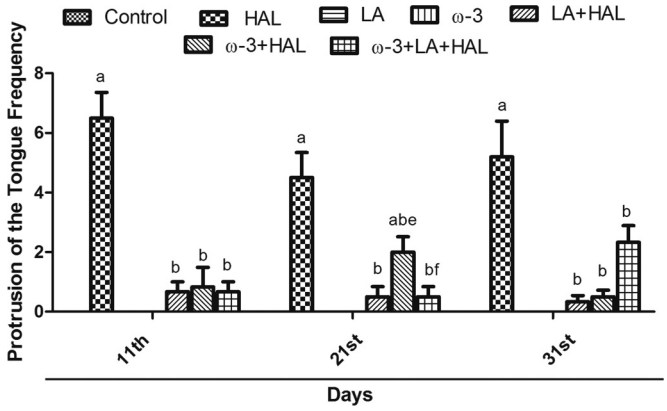
The groups treated of LA alone (11th day: 0.0 ± 0.0; 21st day: 0.0 ± 0.0; 31st day: 0.0 ± 0.0), ω-3 alone (11th day: 0.0 ± 0.0; 21st day: 0.0 ± 0.0; 31st day: 0.0 ± 0.0), and associations groups of LA and HAL (11th day: 0.8 ± 0.4; 21st day: 0.6 ± 0.4; 31st day: 0.2 ± 0.2), ω-3 and HAL (11th day: 0.6 ± 0.5; 21st day: 2.1 ± 0.6; 31st day: 0.4 ± 0.2) or LA, ω-3, and HAL (11th day: 0.4 ± 0.2; 21st day: 0.3 ± 0.2; 31st day: 1.7 ± 0.5) showed a decrease in the number of PT compared with the HAL group in the 3 time periods analyzed (Fig. 4).

Neurochemical study

Concentration of lipid peroxidation (TBARS)

The results showed that chronic exposure of the cells of the PFC, HC, and S to HAL (1480 ± 46.7; 1525 ± 69.9; 1522 ± 53.3, respectively) caused an increase in MDA (μmol MDA/g of tissue) content when

Fig. 4. Determination of the effect of lipoic acid and (or) omega-3 (ω-3) alone or associated with haloperidol (HAL) on the number of tongue protrusions in the test orofacil dyskinesia. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey's test as post hoc test were used. Lowercase letters above bars: a vs. control, b vs. HAL, c vs. LA, d vs. ω-3, e vs. LA + HAL, f vs. ω-3 + LA + HAL, with $p < 0.0001$.



compared with control group (100.6 ± 22.7, 94.6 ± 20.4, 80.4 ± 7.9, respectively) (Figs. 5A, 5B, and 5C).

In the 3 areas studied, the groups treated with LA (PFC: 1041 ± 37.0; HC: 980.6 ± 45.0; S: 1052 ± 59.0) or ω-3 (PFC: 1085 ± 42.5, HC: 1066 ± 53.1, S: 1054 ± 57.6) alone showed a significant reduction in the levels of TBARS compared with the HAL group ($p < 0.0001$). Comparing the associations groups with the group of HAL alone was possible to observe a significant reduction in lipid peroxidation in the TBARS assay in the 3 brain areas (ω-3 + HAL PFC: 834.6 ± 55.6, HC: 780.1 ± 43.7, S: 882.8 ± 77.3; LA + HAL PFC: 1091 ± 41.6, HC: 1067 ± 37.3, S: 1051 ± 57.7; HAL PFC: 1480 ± 46.7; HC: 1525 ± 69.9; S: 1522 ± 53.3) ($p < 0.0001$) (Figs. 5A, 5B, and 5C).

Also, with respect to the associations, the group of ω-3 and HAL presented a greater reduction in MDA levels when compared with the combination of LA and HAL group in the PFC and HC (Figs. 5A and 5B). In contrast, the association group with LA, ω-3, and HAL showed better response in the reduction of lipid peroxidation induced by chronic use of HAL presenting results close to those obtained in the control group in the 3 areas studied (ω-3 + LA + HAL PFC: 211.1 ± 44.0, HC: 101.1 ± 38.9, S: 121.9 ± 43.2) (Figs. 5A, 5B, and 5C).

Nitrite determination

The results showed that the concentrations of nitrite/nitrate (μmol/g of tissue) in the HAL group (2.8 ± 0.0) increased in the PFC group compared with the control group (1.0 ± 0.0). The group of LA alone (1.9 ± 0.1) or ω-3 alone (1.8 ± 0.0) showed an increase in the concentrations of nitrite/nitrate compared with the control group in the PFC and a decrease when compared with the HAL group (Fig. 6A).

All associations, LA and HAL (1.0 ± 0.2), ω-3 and HAL (1.6 ± 0.0), or ω-3 and LA and HAL (1.2 ± 0.1), showed increased concentration of nitrite/nitrate compared with the control group in the PFC and a decrease when compared with the HAL group ($p < 0.0001$) (Fig. 6A).

Also, in relation to concentrations of nitrite/nitrate, the group treated with HAL (1.7 ± 0.0) showed an increase when compared with the control group (0.9 ± 0.0) in the HC. The group of LA alone (0.4 ± 0.0) showed a decrease compared with the HAL group (Fig. 6B).

The group of ω-3 alone (1.6 ± 0.0) as well as the association's groups with LA and HAL (1.6 ± 0.0) or ω-3 and HAL (1.6 ± 0.1) showed increased in the concentrations of nitrite/nitrate compared with the control group in HC. The group of the association with LA, ω-3, and HAL (1.0 ± 0.0) showed a decrease in the concentrations of nitrite/nitrate compared with HAL group in HC (Fig. 6B).

Fig. 5. Effect of α-lipoic acid (LA) and (or) omega-3 (ω-3) on lipid peroxidation in the prefrontal cortex (PFC), hippocampus (HC), and striatum (S) of rats subjected to chronic treatment with haloperidol (HAL). Values represent mean ± SEM of TBARS quantities expressed in micromoles of MDA per gram of tissue. ANOVA and Tukey's test as post hoc test were used. Lowercase letters above bars: a vs. control, b vs. HAL, e vs. LA + HAL, f vs. ω-3 + HAL with $p < 0.0001$.

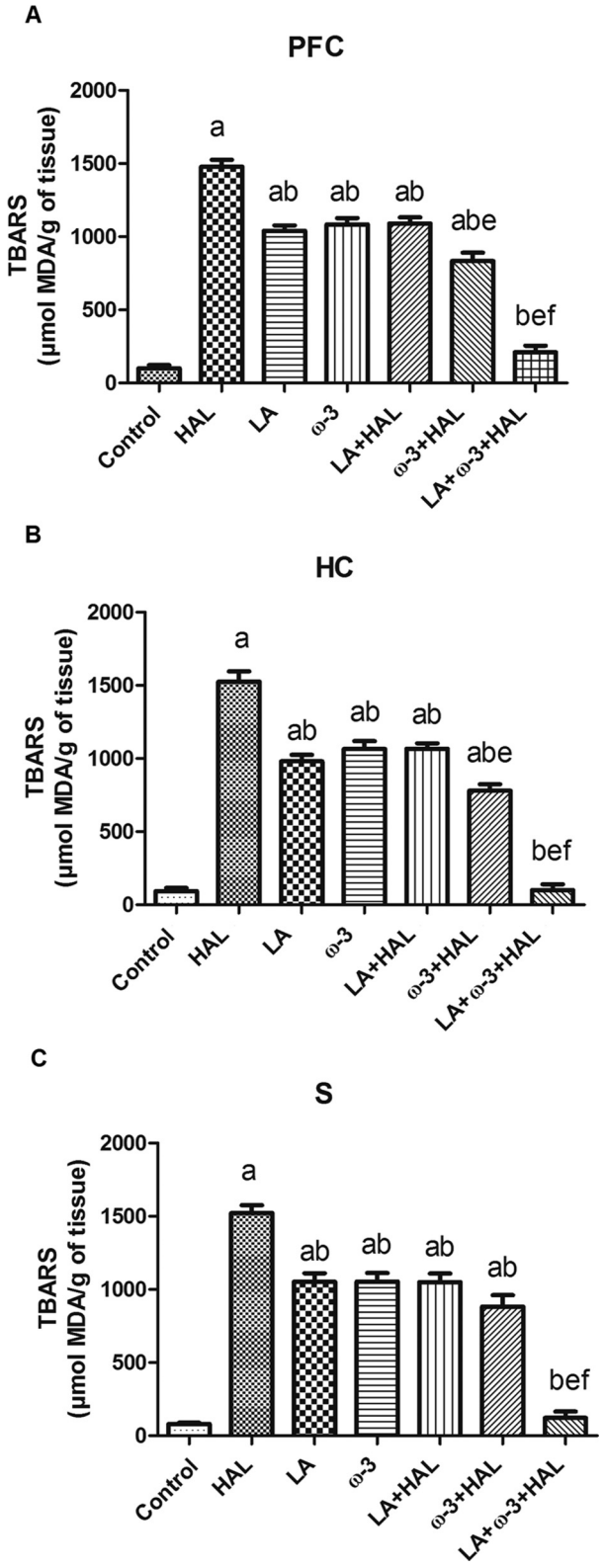
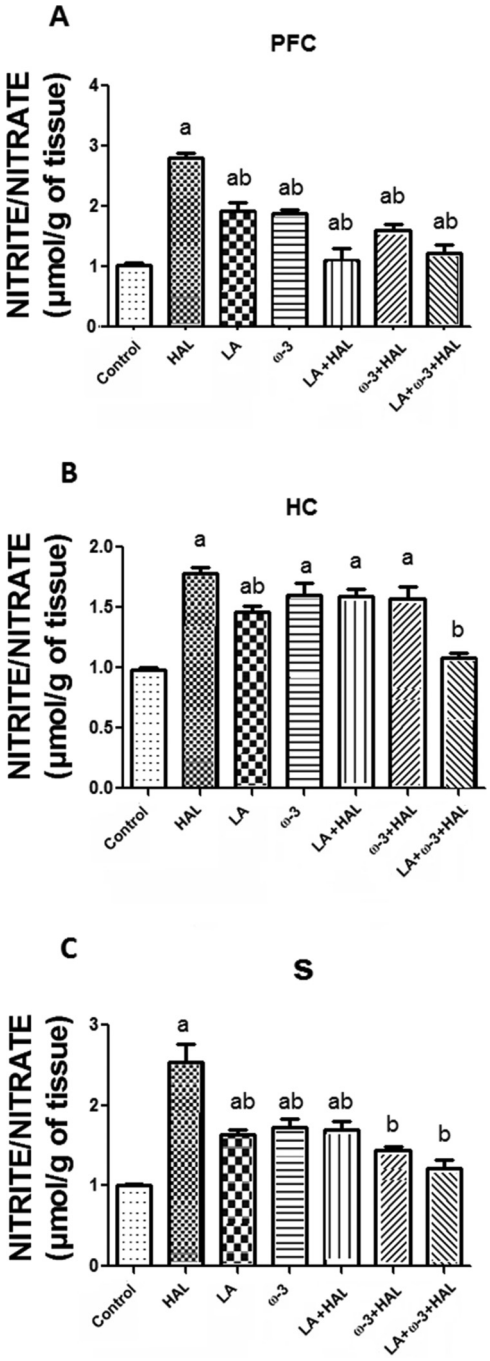


Fig. 6. Effect of α-lipoic acid (LA) and (or) omega-3 (ω-3) on the concentration of nitrite/nitrate in the prefrontal cortex (PFC), hippocampus (HC), and striatum (S) of rats subjected to chronic treatment with haloperidol (HAL). Values represent mean ± SEM of the amounts of nitrite/nitrate expressed in micromoles per gram of tissue. ANOVA and Tukey's test as post hoc test were used. Lowercase letters above bars: a vs. control, b vs. HAL, with $p < 0.0001$.



In the S, the concentrations of nitrite/nitrate in the HAL group (2.5 ± 0.2) increased compared with the control group (1.0 ± 0.0). The groups treated with LA alone (1.6 ± 0.0) or ω-3 alone (1.7 ± 0.1) or association group treated with LA and HAL (1.7 ± 0.1) showed increased concentrations of nitrite/nitrate compared with the control group and decreased when compared with the HAL group in S (Fig. 6C).

The associations with ω-3 and HAL (44.0 ± 0.0) or LA, ω-3 and HAL (1.2 ± 0.1) exhibited decreased of concentrations of nitrite/nitrate compared with the HAL group (Fig. 6C).

Discussion

The open-field test aims to study the action of the dopamine system, serotonergic and noradrenergic emotional and exploratory behavior through the horizontal scanning (mobility), vertical (rearing), and self-cleaning (grooming). It is widely used as an evaluation tool of the substances action that may act on these neurotransmitter systems promoting motor abnormalities (bradykinesia or hyperlocomotion) and emotional (anxiety) (Schallert et al. 2000).

The results showed that LA and ω-3 promoted improvement in motor performance in the open-field test. This improves motor due to the antioxidant effect of these substances, reflecting on monoamine levels. The LA has the capacity to alter the levels of monoamines and their metabolites by stimulating the synthesis, release in the synaptic cleft and reduced metabolism of these neurotransmitters, thereby increasing its availability in the central nervous system (Chng et al. 2009; Santos et al. 2010).

The LA and ω-3 were able to reduce the time spent on the bar in the catalepsy test, indicating a possible neuroprotective effect. This effect is related to the ability of these substances to control the state oxidant/antioxidant, stabilizing the membranes of brain tissue structures and acting as antiapoptotic (Barcelos et al. 2010; Thaakur and Himabindhu 2009).

The typical neuroleptics promote blocking dopamine D2 receptors; this results in up-regulation of these receptors in the dopamine-rich areas, such as the basal ganglia. The increase in catecholamine levels results in the overproduction of ROS. The HAL reduces gene expression of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GHP), thus reducing the complex antioxidant defense (Thaakur and Himabindhu 2009).

The LA and ω-3 promote a reduction in the number of VCM and PT induced by HAL, indicating a possible neuroprotection. This effect is due to the reduction in ROS levels as well as increased content of SOD, CAT, and GHP. Studies have shown that D2 receptor blockade is related to increased glutamate release in the striatum. This increase in glutamate promotes neurotoxicity in the striatum resulting in extrapyramidal events. This effect is related to the chronic use of haloperidol. (Bošković et al. 2016; Naidu et al. 2003; Thaakur and Himabindhu 2009).

The nervous system is more sensitive to the damaging action of free radicals than other tissues of the organism once the metabolism of the brain is extremely high which favors the continual formation of reactive oxygen species and nitrogen and the antioxidant defense system is not as effective (Ferreira and Abreu 2016).

Evidence suggests that oxidative stress plays an important role in the pathophysiology of TD, a fact proven by studies published about this topic (Aguiar et al. 2010; de Oliveira et al. 2013).

In this research, the HAL increased the concentration of nitrite/nitrate in the striatum. Another interesting result is that oxidative damage was not restricted to this area of the brain, but also mostly affected the prefrontal cortex and hippocampus. This demonstrates that the changes produced by chronic use of HAL are not limited only to the neurons of the striatum.

As an antioxidant, LA and ω-3 work by removing the hydroxyl radicals, hydrogen peroxide in its free form, superoxide and peroxynitrite (de Araújo et al. 2011; Bošković et al. 2016). Because of this potent effect, LA and ω-3,would also be able to prevent neuronal damage caused by reactive species derived from oxygen and nitrogen (Bošković et al. 2016; Maczurek et al. 2008; Thaakur and Himabindhu 2009).

The ω-3 plays a fundamental role in maintaining neuronal integrity to promote brain development and synaptic plasticity. Besides ω-3 present a possible antioxidant effect by promoting the removal of ROS or interfere with the production of ROS. The EPA and DHA can alter production of catecholamines like dopamine and serotonin, which are fundamental for the maintenance of motor function controlled by the dopaminergic system in the striatum (Cardoso 2009).

Lipid peroxidation is the broader process of oxidative damage by promoting the breakdown of the cell membrane lipids and the formation of the peroxyl radical. Once this event is started, this spreads, inducing cell destruction chain (Ferreira and Abreu 2016).

LA and ω-3 showed a possible neuroprotective effect because it was able to significantly reduce the lipid peroxidation in the TBARS assay in the striatum, prefrontal cortex, and hippocampus.

The EPA and DHA to be metabolized by cyclooxygenase-2 (COX-2) resolvin produce the E and D series, respectively. The DHA can be converted to protectine D1 and D1 neuroprotectine by the action of lipoxygenase (LOX). The neuroprotectine D1 is produced in response to oxidative damage in the brain, serving as anti-inflammatory and antioxidant (Cardoso 2009).

Conclusion

The LA and the ω-3 were able to promote an improvement of motor function observed through the open-field test and reduced the time spent on the bar in the catalepsy test and the number of empty movements of chewing and PT in the orofacial dyskinesia test. They also were able to promote a reduction in the concentrations of nitrite/nitrate as well as HAL-induced lipid peroxidation in 3 brain areas investigated. Such a response would be related to the antioxidant and anti-inflammatory effect of these substances, suggesting a neuroprotective action against extrapyramidal injury induced by chronic use of HAL.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

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