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OMEGA-3 PREVENTS BEHAVIOR RESPONSE AND BRAIN OXIDATIVE DAMAGE IN THE KETAMINE MODEL OF SCHIZOPHRENIA

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Abstract—Supplementation with omega-3 has been identified as an adjunctive alternative for the treatment of psychiatric disorders, in order to minimize symptoms. Considering the lack of understanding concerning the pathophysiology of schizophrenia, the present study hypothesized that omega 3 prevents the onset of symptoms similar to schizophrenia in young Wistar rats submitted to ketamine treatment. Moreover, the role of oxidative stress in this model was assessed. Omega-3 (0.8 g/kg) or vehicle was given by orogastric gavage once daily. Both treatments were performed during 21 days, starting at the 30th day of life in young rats. After 14 days of treatment with omega-3 or vehicle, a concomitant treatment with saline or ketamine (25 mg/kg ip daily) was started and maintained until the last day of the experiment. We evaluated the pre-pulse inhibition of the startle reflex, activity of antioxidant systems and damage to proteins and lipids. Our results demonstrate that supplementation of omega-3 prevented: decreased inhibition of startle reflex, damage to lipids in the hippocampus and striatum and damage to proteins in the prefrontal cortex. Furthermore, these changes are associated with decreased GPx in brain tissues evaluated. Together, our results suggest the prophylactic role of omega-3 against the outcome of symptoms associated with schizophrenia.

Key words: schizophrenia, prepulse inhibition, ketamine, omega 3, antioxidants.

INTRODUCTION

The major structural components of the phospholipid membrane are polyunsaturated fatty acids (PUFAs), denominated as essential fatty acids for not being synthesized by the body from the fatty acids de novo synthesis (Reddy et al., 2004; Peet, 2008). Specifically, the omega-3 fatty acids family such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are polyunsaturated carboxylic acids, in which the insaturation is present at the third carbon from the end opposite the carboxyl (Youdim et al., 2000). In humans, omega-3 fatty acids are required to maintain under normal conditions, cell membranes, brain function and the transmission of nerve impulses. In addition, these fatty acids facilitate the transfer of atmospheric oxygen to the blood plasma, favor the hemoglobin synthesis and contribute to cell division processes (Youdim et al., 2000; Yehuda et al., 2002).

Given the variety of functions performed by omega-3, the phospholipid hypothesis of schizophrenia was developed, suggesting that several issues that affect mental health can be the result of abnormalities of phospholipid structure of neuronal membranes (Horrobin et al. 1994; Peet, 2008). In this regard, a landmark study using omega-3 supplementation in schizophrenic patients indicated a significant improvement in symptoms of schizophrenia as in tardive dyskinesia (Laugharne et al., 1996). Likewise, such benefits were reported in a double blind placebo-controlled trials (Amminger et al., 2010). Furthermore, these initial studies indicated that EPA was more effective than DHA (Peet et al., 2001). Moreover, a study showed the beneficial effect of the omega-3 supplementation on positive and negative symptoms of schizophrenia as well as the severity of side effects induced by haloperidol (Sivrioglu et al., 2007). Recently, a study showed that omega-3 can reduce the symptoms of schizophrenia for its neuroprotective properties without presenting clinically relevant adverse effects. Also, the study indicates that omega-3 helps to reduce the progression of psychotic disorder (Amminger et al.,

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Abbreviations: ANOVA, analysis of variance; BDNF, Brain-derived neurotrophic factor; CAT, catalase; DHA, docosahexaenoic acid; DNPH, dinitrophenylhydrazine; EDTA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; GPx, glutathione peroxidase; GSH, glutathione; NADPH, dihydronicotinamide-adenine dinucleotide phosphate; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive species.

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2010). On the other hand, others studies indicated no evidence of omega-3 benefits for schizophrenia (Fenton et al., 2001; Hibbeln et al., 2003). Therefore, these data indicate that additional studies need to be performed to establish the role of omega-3 in schizophrenia.

Schizophrenia is a chronic disorder psychiatric that induces positive, negative and cognitive symptoms. For reproducing in animals some of the symptoms of schizophrenia observed in humans, ketamine is used (Park and Holzman, 1992; Newcomer, 1999; Becker and Grecksch, 2004). Behavioral changes induced by ketamine in rats that are related to pathophysiology of schizophrenia as: hyperlocomotion, stereotypy, impaired information processing with cognitive functions of memory and attention, and impaired social interaction (Lipska and Weinberber, 2000; Bubeníková-Valesová and Horácek, 2010).

Although the etiology of schizophrenia is not clear in the literature, current research indicates that stress due to the accumulation of reactive oxygen species (ROS) is associated with the pathophysiology of the disease (Ciobica et al., 2011; Yao and Keshavan, 2011; Ruiz-Litago et al., 2012). The presence of oxidative stress occurs by increased levels of ROS, reactive nitrogen species (RNS) or by an imbalance in the activity of endogenous antioxidant systems (antioxidant enzymes) (Kwon et al., 2003; Berg et al., 2004). As a consequence of the increase in ROS and the failure of the endogenous antioxidant system a damage to DNA, proteins and membrane lipids can occur (Konat, 2003; Kwon et al., 2003).

Studies indicated that schizophrenic patients showed reduction of total glutathione (GSH) (Do et al., 2000) and N-acetyl-cysteine (NAC) might ameliorate this redox dysregulation (Carmeli and Knyazeva, 2012). Another study indicated that the levels of the antioxidant enzyme glutathione peroxidase (GPx) decreased both in treated and untreated patients (Miljevic et al., 2010). Also, increased levels of markers of lipid peroxidation were observed in similar population (Dadheech et al., 2008; Padurariu et al., 2010). Similarly, an experimental study produced by our group suggests that the animal model of schizophrenia induced by ketamine induces changes in the activity of superoxide dismutase (SOD), catalase (CAT) and GPx, resulting in protein and lipid damage (de Oliveira et al., 2009). Moreover, das Neves Duarte et al. (2012) showed that a glutamate-cysteine ligase modulatory subunit-knockout mice (mouse model with chronic GSH deficit) presents a redox imbalance, similar to those reported in early schizophrenia.

Considering the lack of understanding concerning the pathophysiology of schizophrenia and the lack of effective pharmacological treatments available, studies that focus on both the discovery of new therapeutic strategies as well as mechanisms associated with the disease are of extreme relevance. In this regard, the present study hypothesized that omega 3 prevents the onset of symptoms similar to schizophrenia in adult Wistar rats submitted to ketamine treatment and that this effect is mediated by the action of antioxidant systems. Furthermore, the chosen parameters were evaluated 1 h after the last injection of ketamine, a glutamatergic antagonist widely used in experimental research focused on the study of schizophrenia.

EXPERIMENTAL PROCEDURES

The animals included in the study were handled according to the NIH Guide for the Care and Usage of Laboratory Animals and according to the rules of the Brazilian Society for Neuroscience and Behavior (SBNeC). Furthermore, the procedures were submitted and approved by the ethics committee of Universidade do ExtremoSulCatarinense (UNESC). The rats were randomized and the biochemical and behavioral experiments were double-blind controlled.

Animals

A total of 60 male Wistar rats with 30 days of age and weight around 80–150 g were included in the study. The rats were obtained from colonies of the bioteriumfrom Universidade do ExtremoSulCatarinense. The animals were kept in climate-controlled (22 °C) condition with light–dark cycle of 12 h and received food and water *ad libitum* throughout the experiment. The choice of males occurred because females present major variability response in both behavioral and biochemical tests due to the specific hormonal profile.

Omega 3 supplementation and animal model of schizophrenia

Omega-3 PUFAs at a dose of 0.8 g/kg was given by orogastric gavage (oral route) once daily. For the vehicle, an inert oil with no impact on omega-3 fatty acid metabolism was chosen. The vehicle was administered by gavage at the same concentration as the omega-3 PUFAs. Both treatments (vehicle or omega-3) started in young animals at the 30th day of life with a total period of 21 days. Starting from the 14th day, the groups were subdivided for the second treatment with saline or ketamine during 7 days.

At the end of the experiment a number of 60 animals performed four groups, as follows: (1) vehicle plus saline (called Tween + Sal group, $n = 15$), (2) vehicle plus ketamine (called Tween + Ket group, $n = 15$), (3) omega-3 plus saline (called omega + Sal, $n = 15$), (4) omega-3 plus ketamine (Omega-3 + Ket, $n = 15$). Four groups of comparison were required due to the oily nature of the omega-3 oily and the aqueous nature of ketamine. In this type of pharmacological experiment, for each active substance there is a need of an inert substance similar in nature to a placebo. The supplementation of omega-3 was performed using capsules of fish oil containing EPA (18%) and DHA (12%). Ketamine (CU ChemieUetikon, Germany) was used at a dose of 25 mg/kg intraperitoneally for 7 days as an animal model of schizophrenia. The volume of ketamine injections was prepared at a volume of (CU ChemieUetikon, Germany), prepared in saline at a volume of 1 mL/100 g (Becker and Grecksch, 2004; Imre et al., 2006; Tomiya et al., 2006). The administration of 25 mg/kg was used to mimic some