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Effects of omega-3 supplementation on interleukin and neurotrophin levels in an animal model of schizophrenia

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ABSTRACT

New studies suggest that polyunsaturated fatty acids, such as omega-3, may reduce the symptoms of schizophrenia. The present study evaluated the preventive effect of omega-3 on interleukines (IL) and neurotrophin brain-derived neurotrophic factor (BDNF) levels in the brains of young rats subjected to a model of schizophrenia. Treatment was performed over 21 days, starting on the 30th day of rat's life. After 14 days of treatment with omega-3 or vehicle, a concomitant treatment with saline or ketamine (25 mg/kg) was started and maintained until the last day of the experiment. BDNF levels in the rat's prefrontal cortex were decreased at 1 h and 24 h after the last administration of ketamine, whereas the group administered with ketamine and omega-3 showed a decrease in BDNF levels only after 24 h. In contrast, both interventions induced similar responses in levels of IL-1β and IL6. These findings suggest that the similarity of IL-1β and IL6 levels in our experimental groups is due to the mechanism of action of ketamine on the immune system. More studies have to be carried out to explain this pathology. In conclusion, according to previous studies and considering the current study, we could suggest a prophylactic role of omega-3 against the outcome of symptoms associated with schizophrenia.

Key words: omega-3, ketamine, schizophrenia, neurotrophins, interleukins.

INTRODUCTION

Schizophrenia is considered one of the most severe psychiatric disorders, affecting approximately 1% of the world population (Andreasen et al. 2000). The lack of clarification about the pathophysiology of the disorder complicates treatment strategies (Tajima et al. 2009). Schizophrenia is usually treated with a combination of psychotherapy and social settings, as well as the administration of drugs, including atypical and typical antipsychotics (Kapur and Remington 2001). Given the

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information presented, it is clear that the currently available pharmacological treatments for patients with schizophrenia are far from ideal. Moreover, despite the growing consensus that schizophrenia is a neural disorder, its etiology, neuropathology, pathophysiology, psychopharmacology and genetics open a wide field of research. In this context, basic animal research is a promising tool for studying the neurobiological basis of the neural and behavioral disorders relevant to schizophrenia, providing the basis for the evaluation and development of new therapies (Meyer and Feldon 2010).

Current studies utilize pharmacological tools to evaluate the effect of new protective compounds, such as omega-3, against to schizophrenia. A widely used animal model of schizophrenia involves the acute or repeated administration of ketamine (Becker and Grecksch 2004, Bubeníková-Valesová et al. 2008, Canever et al. 2010, De Oliveira et al. 2011).

According to Horrobin (1998), studies of the membrane phospholipid composition (MPC) hypothesis of schizophrenia, argue that alterations in the MPC of the brain, as a direct result of changes in fatty acid levels, could be involved in the etiology of schizophrenia. This argument is based on findings of reduced omega-3 and omega-6 polyunsaturated fatty acids (PUFA) and abnormalities in phospholipid metabolism in schizophrenia (Arvindakshan et al. 2003a, b). It has been argued that dysfunctional fatty acid metabolism could be involved in the etiology of schizophrenia, based on the findings of reduced omega-3 polyunsaturated fatty acids (PUFAs) in individuals deemed to be at an ultra high risk for psychotic disorders (Amminger et al. 2010). This is due to their altering effect on membrane fluidity and receptor responses following their incorporation into the cell. Omega-3 also can interact with the dopaminergic and serotonergic systems, which have been associated with the pathophysiology of schizophrenia.

Thus, evidence suggests that PUFA, such as long chain omega-3, may reduce symptoms of schizophrenia due to their neuroprotective properties without, however, presenting clinically relevant adverse effects. In general, fatty acid treatment seems to be a good strategy for the prevention of psychosis in individuals who are at increased risk of the disorder (McGorry et al. 2008, Morrison et al. 2004, Amminger et al. 2010), suggesting that the omega-3 may have neuroprotective or anti-inflammatory properties. In addition to the evidence presented, many recent trials have supported the hypothesis of the role of inflammation and nutritional deficiencies in the pathogenesis of schizophrenia (Raffa et al. 2011).

It is known that schizophrenia is associated with increased levels of certain inflammatory markers, suggesting the possibility that the disorder is primarily an inflammatory disease (Peet and Stokes 2005, Peet 2006). A meta-analysis of 62 studies with 2298 people with schizophrenia and 1858 healthy volunteers was performed to verify the cytokine imbalances in schizophrenia (Potvin et al. 2008). IL-1β, IL-6, and transforming growth factor-beta (TGF-β) were significantly increased in first episode and acutely relapsed patients and were state biomarkers (Monji et al. 2009). Meisenzahl et al. (2001), described the relationship between the loss of brain volume and increased production of immunological markers, like IL-1 (interleukin 1). Similarly, Garver et al. (2003) presented the morphological changes of the brain volume and increased levels of IL-6 in the CSF (cerebrospinal fluid) in schizophrenia.

Currently, there is growing recognition that the pathophysiology of schizophrenia may be a result of dysregulated synaptic plasticity with changes in neurotrophins. The neurotrophin brain-derived neurotrophic factor (BDNF) is widely distributed in the CNS and is considered a crucial protein in psychiatric illness. Thus, evidence from experimental studies indicates the role of diet, social and family interactions in the regulation of BDNF (Salum et al. 2008). High levels of BDNF

have been detected in human blood platelets, suggesting that human platelets may provide an important source of BDNF to peripheral sensory neurons during regeneration at the site of nerve damage (Radka et al. 1996, Yamamoto and Gurney 1990).

The pharmacological interventions for schizophrenia promote positive outcomes, but at the same time trigger side effects that limit the effectiveness of treatment and compromise the patients' quality of life. Thus, studies that can identify adjuvant strategies are very important for this population (Amminger et al. 2010). Likewise, the identification of biochemical markers associated with new strategies contributes to our knowledge about the pathophysiology and treatment of the disease. In this sense, we evaluated the preventive effect of omega-3 on the levels of pro-inflammatory interleukins and BDNF in the brains of young rats subjected to the ketamine-induced model of schizophrenia.

Significant Outcomes

- Omega-3 demonstrated similar beneficial effects in the IL-1β and IL6 levels in an animal model of schizophrenia.
- Omega-3 demonstrated significant effects on BDNF levels in the animals' brains.

Limitations

Other immunological parameters could be dosed

We have just assayed 1 and 24 h we could find alterations in other times.

MATERIALS AND METHODS

All procedures used in the present study complied with the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals that follows the "Principles of laboratory animal care". All experimental protocols were designed with the goal of keeping the number of animals used to a minimum, as well as minimizing their suffering. These protocols were conducted in accordance with national and international legislation (guidelines of Brazilian Council of Animal Experimentation – CONCEA – and of the U.S. Public Health Service's Policy on Human Care and Use of Laboratory Animals—PHS Policy), and with the approval of the Ethics Committee for Animal Research of the Universidade do Extremo Sul Catarinense (UNESC) Protocol 14/2012.

Animals

Young male Wistar rats were selected for this study from the Bioterio of Universidade do Extremo Sul Catarinense (UNESC) (aged 30-days, weight 80-150 g). The animals were maintained in a 12 h light–dark cycle (lights on at 6:00 a.m.) at constant room temperature (22 ± 2 ºC), humidity (60 ± 75%) and were housed in groups of five animals per cage (49 x 34 x 16 cm) with free access to food and water.

Omega-3 Supplementation and the Animal Model of Schizophrenia

Omega-3 PUFAs (0.8 g/kg) were given by orogastric gavage once daily. For the vehicle, 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) were started in young animals at the 30th day of life for a total period of 21 days. Starting from the 14th day, the groups were subdivided for the second treatment with saline or ketamine for 7 days.

At the end of the experiment we used four groups, as follows: 1) vehicle plus saline**,** 2) omega plus saline, 3) vehicle plus ketamine, and 4) omega plus ketamine. The omega-3 supplementation was performed using fish oil capsules containing EPA (18%) and DHA (12%). Ketamine (25 mg/kg; CU Chemie Uetikon, Germany) was administered intraperitoneally for 7 days as an animal model of schizophrenia. The volume of the ketamine injections was prepared in saline at a volume of 1

mL/100 g (Becker and Grecksch 2004, Imre et al. 2006, Tomiya et al. 2006).

Thirty minutes after the last injections, the rats were euthanized by decapitation and the brain structures (striatum, hippocampus and prefrontal cortex) were carefully dissected for biochemical analysis.

Biochemical Analysis

BDNF and interleukin (IL-1β, IL6) levels in the prefrontal cortex, hippocampus and striatum were measured by sandwich ELISA according to the manufacturer's instructions (Chemicon, USA for BDNF and Millipore, USA and Canada for NGF). Briefly, brain structures were homogenized in a phosphate buffered saline solution (PBS) with a protease inhibitor cocktail (Sigma). Microtiter plates (96-well flat bottom plates) were coated for 24 h with samples diluted 1:2 in sample diluent using a standard curve varying from 7.8 to 500 pg/ml BDNF and ILs. The plates were then washed four times with sample diluent, and a specific monoclonal antibody for each protein (IL6 and IL-1β or BDNF) was added. After washing, a peroxidase-conjugated secondary antibody was added to each well and incubated at room temperature for 1 h. After addition of streptavidin-enzyme, the concentration of interleukins and BDNF was determined by absorbance at 450 nm. The standard curve demonstrates a direct relationship between the optical density (OD) and the concentration. The total protein concentration was measured using bovine serum albumin as a standard, as previously described by Lowry et al. (1951).

It is noteworthy that the pro-inflammatory response is observed later in similar models (Strous and Shoenfeld 2006). In contrast, changes in the levels of BDNF are observed both immediately after the intervention and later **(**Reus et al. 2011**)**. Therefore, we chose to evaluate the levels of interleukin 24 h after death and BDNF both at 1 hand 24 h after death.

Statistical Analysis

All values are expressed as the means ± S.E.M. (n equals the number of rats included in each analysis). The statistical analysis was carried out using two-way analysis of variance (ANOVA) with pretreatment (vehicle vs. omega) and treatment (vehicle vs. ketamine) as independent variables. The accepted level of significance for the tests was ≤ 0.05. All tests were performed using the Statistics® software package (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Our study tested the hypothesis that the omega-3 polyunsaturated fatty acid prevents biochemical changes in animals subjected to the ketamine-induced animal model of schizophrenia. Figure 1 shows the levels of interleukin 1β at 24 h after the last injection of ketamine in the different cerebral structures studied. Statistical analysis indicates that the two categorical variables (omega and/or ketamine) did not demonstrate effects on this parameter. A similar response was demonstrated for the levels of IL-6 after 24 h, as shown in Figure 2. Thus, both interventions induced similar responses in the levels of IL-1β and IL6 at 24 h after the last administration of ketamine. Data on the concentrations of BDNF in Figure 3 indicate that the striatum and hippocampus showed no changes with omega-3 or ketamine. In contrast, the levels of BDNF were diminished in the ketamine-treated group compared to the control group (vehicle + saline, $p < 0.05$). Figure 3 shows the results of dosage on BDNF 1h after the last injection of ketamine, indicating an acute response to the interventions. Furthermore, the two-way ANOVA indicated an effect of ketamine on this parameter [$F(1,11) = 6.66$, $p < 0.05$]. Figure 4 shows the results for BDNF concentrations in the brain structures at 24 h after the last administration of ketamine. Likewise, only the prefrontal cortex was affected for this parameter. Both ketamine-treated groups

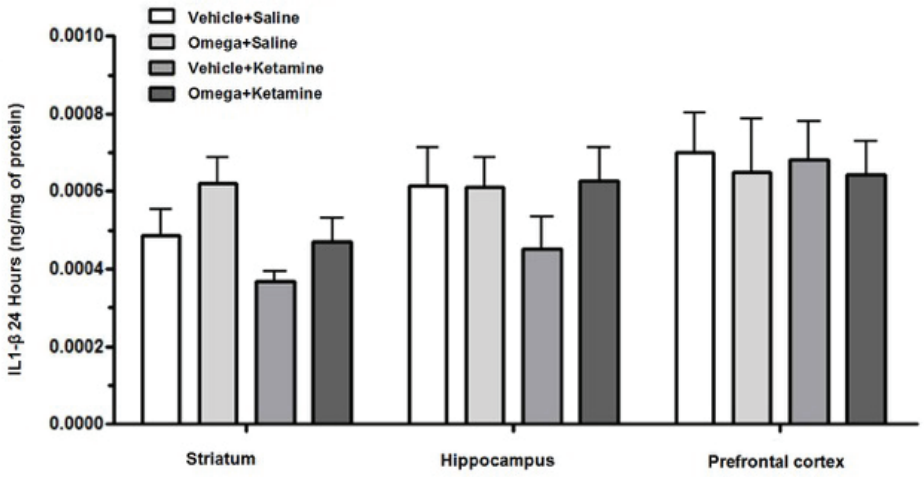

Figure 1 - Effect of omega-3 supplementation and/or ketamine treatment (25 mg/kg) on the levels of IL1-β in different rat brain structures at 24 h after the last administration of ketamine. Data are expressed as the mean ± SEM for five animals in each group. (White bars = vehicle + saline, light gray bars = omega + saline, medium gray bars = vehicle + ketamine and dark gray bars = omega + ketamine).

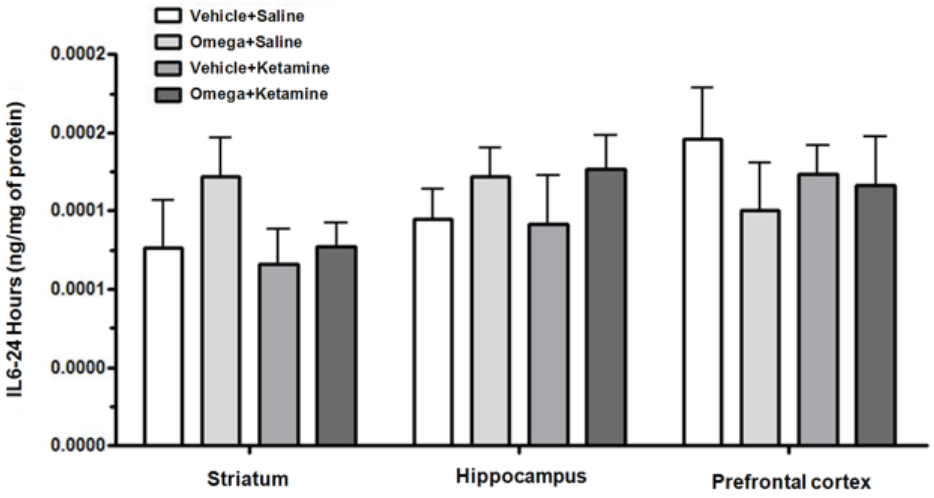

Figure 2 - Effect of omega-3 supplementation and/or ketamine treatment (25 mg/kg) on the levels of IL6 in different rat cerebral structures at 24 h after the last administration of ketamine. Data are expressed as the mean ± SEM for five animals in each group. (White bars = vehicle + saline, light gray bars = omega + saline, medium gray bars = vehicle + ketamine and dark gray bars = omega + ketamine).

(vehicle+ketamine; ketamine+omega) showed decreased levels of BDNF compared to the vehicle+saline ($p<0.01$) and omega+saline groups ($p<0.05$), respectively. Therefore, the delayed action of ketamine in reducing the concentrations of BDNF in the brain [$F(1,12) = 21.92$, $p<0.01$] highlighted a selective effect of ketamine on the prefrontal cortex.

DISCUSSION

Omega-3 may be involved in the etiology of schizophrenia because biochemical studies showed

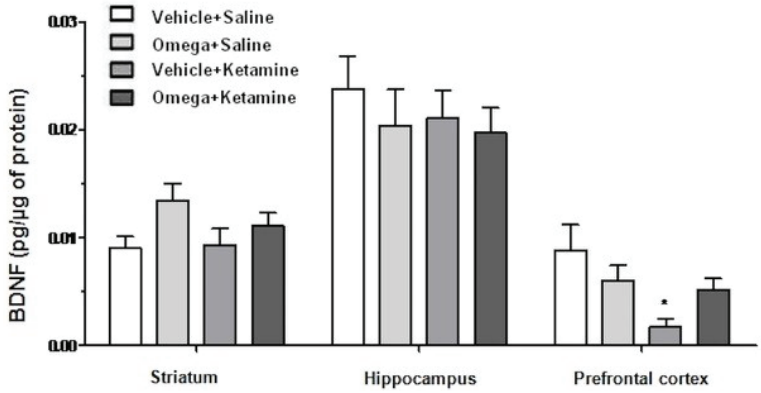

Figure 3 - Effect of omega-3 supplementation and/or ketamine treatment (25 mg/kg) on BDNF levels in different rat brain structures at 1 h after the last administration of ketamine. Data are expressed as the mean ± SEM for five animals in each group. * Different from vehicle + saline (white bars = vehicle + saline, light gray bars = omega + saline, medium gray bars = vehicle + ketamine and dark gray bars = omega + ketamine).

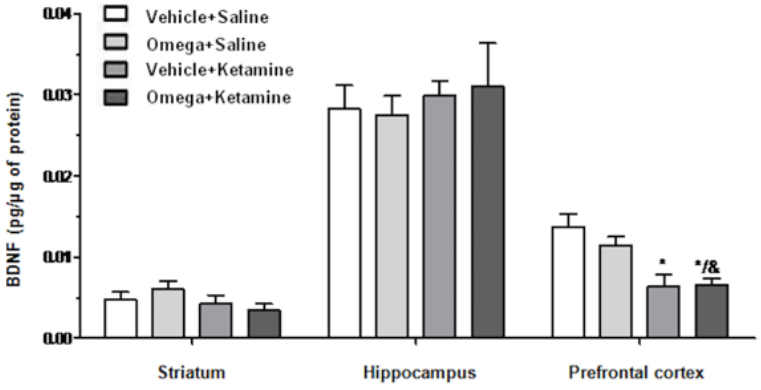

Figure 4 - Effect of omega-3 supplementation and/or ketamine treatment (25 mg/kg) on BDNF levels in different rat brain structures at 24 h after the last administration of ketamine. Data are expressed as the mean ± SEM for five animals in each group. * Different from vehicle + saline (white bars = vehicle + saline, light gray bars = omega + saline, medium gray bars = vehicle + ketamine and dark gray bars = omega + ketamine). */& Different from vehicle + omega 3 (white bars = vehicle + saline, and medium gray bars = vehicle + ketamine).

low levels of omega-3 PUFA in the red blood cells of patients with major depressive disorder or schizophrenia. EPA and docosahexaenoic acid (DHA), the two main omega-3 fatty acids in fish oil, have an important role in the CNS. There is some evidence that the administration of EPA can accelerate the response to treatment, improving tolerability of antipsychotic medications (Berger et al. 2007). Omega-3 fatty acids are thought to have some effect on inflammation by modulating the amount and types of eicosanoids produced. Other effects are elicited by eicosanoid-independent mechanisms, including actions upon intracellular

signaling pathways, transcription factor activity, and gene expression (Simopoulos 2002).

Currently, research has emphasized the role of neuroinflammation in schizophrenia. There is evidence relating subclinical chronic inflammation and schizophrenia in individuals, usually in their adulthood, who have already developed the illness. Furthermore, other studies supporting immune challenge data show that a dysfunctional immune response is evident in schizophrenia and may play a pivotal role in the pathophysiology of this illness (Doorduin et al. 2009).

There are several competing hypotheses for immune alterations. One hypothesis describes activated microglial cells in the CNS that release pro-inflammatory cytokines to promote neuronal changes (neurogenesis and degradation) that contribute to the pathophysiology of schizophrenia (Monji et al. 2009). Another theory posits that abnormalities of the CNS metabolism arise in schizophrenia due to genetically modulated inflammatory reactions that damage the microvascular system of the brain in reaction to environmental stimuli (Hanson and Gottesman 2005).

Doorduin et al. (2009) demonstrated a neuroinflammatory process in the hippocampus of seven patients recovering from a psychotic episode using PET scan. This suggested that neuroinflammation is an important part of schizophrenia, particularly during psychosis.

There is a reported association of IL-6 with the acute symptoms in bipolar disorder and schizophrenia (Brietzke et al. 2009, Naudin et al. 1996). This comparison involved patients in the non-acute stage of schizophrenia and concluded that there is an increased presence of IL-6 in schizophrenia, providing further evidence that there is chronic immune activation and inflammation in schizophrenia (Potvin et al. 2008).

In contrast, human studies and our ketamine-induced animal model demonstrated that omega-3 supplementation did not induce changes in the studied interleukins (IL1β, IL6) measured at 24 h after the last injection of ketamine. However, 30 mg/kg ketamine showed an increase in locomotor activity, behavior, movement detachment of the head and biochemical changes in some brain tissues, indicating a limitation of the model and significant changes in the immune system (Macedo et al. 2012).

On the other hand, human PET studies measuring the levels of dopamine following ketamine administration suggest that NMDA antagonists increase the release of dopamine in the striatum, and these effects are suppressed, at least in part, by antipsychotic drugs (Corbett et al. 1995, Irifune et al. 1995, Smith et al. 1998). In a recent study, Becker and Grecksch (2004) showed that 5 days of ketamine administration causes changes in dopaminergic, serotonergic and glutamatergic neurotransmission, producing an increase in the D_2 receptor in the hippocampus, a decrease in the frontal cortex glutamate receptor, in addiction to an increase in the dopamine transporter in the striatum and the serotonin transporter in the striatum, hippocampus and frontal cortex.

Inflammation is an important component in maintaining the homeostasis of the organism. However, an inflammatory response may be associated with loss of the homeostasis, causing damage to the tissue or organ dysfunction. Some drugs interact with the inflammatory response in a positive, negative, or "double" manner. Among these drugs, ketamine appears to have a significant positive effect on the regulation of inflammation. The NMDA receptor antagonist operates at different levels of inflammation by mobilizing inflammatory cells, producing cytokines, inflammatory mediators and through regulation. These interactions confer anti-inflammatory properties to ketamine, limiting the exacerbation of systemic inflammation without affecting the local healing processes. This evaluation leads to a complete view of the immunomodulatory properties of this complex anesthetic (Loix et al. 2011).

These findings suggest that the similarity of IL1β and IL6 levels in our experimental groups

are due to the mechanism of action of ketamine on the immune system. Thus, we emphasize the need for studies with new models of schizophrenia to analyze the changes in the levels of these proteins in the immune system. Therefore, further studies should thoroughly evaluate the role of pro-inflammatory cytokines and autoimmunity in the pathophysiology of this mental illness. This will be able to drive strategies for pharmacological interventions that are more targeted and effective for the treatment of schizophrenia, which causes severe impairment in quality of life of the patient and his or her family (Bonhomme et al. 2011).

BDNF plays an important role in neurodevelopment and neural plasticity. A recent meta-analysis concluded that the levels of this neurotrophin are altered in schizophrenic patients (Favalli et al. 2012). A previously described study showed that omega-3 fatty acids can increase BDNF levels and it would be involved in the activation of metabolic pathways (Wu et al. 2004, Balanzá-Martínez et al. 2011). Omega-3 is known to play a role in the regulation of neurotrophins like BDNF and its receptor Trk-B, which are involved in spatial learning and memory (Bhatia et al. 2011). Several studies have reported enhanced hippocampal neurogenesis along with increased levels of BDNF levels following omega-3 PUFAs treatment (Blondeau et al. 2009, Venna et al. 2009).

Experiments conducted in animals have shown an increase of BDNF in the brain during spatial learning. In clinical depression, successful antidepressant treatment increased BDNF levels in serum (Kaneda et al. 2009). Research has also shown that ketamine is capable of significantly increasing BDNF levels in the hippocampus of animals 30 min after administration, but not 24 h after application (Autry et al. 2011).

In the present study, we chronically administered ketamine to cause nerve damage, and the results indicated that ketamine reduced BDNF levels in the prefrontal cortex at 1 and 24 h after treatment. Still, this decrease was not prevented by omega-3 intake for 21 days. This result corroborates previous studies demonstrating that preventing BDNF reduction requires more intense treatment. It is possible that increasing the dose or duration of treatment with omega-3 may influence changes in this marker (Molteni et al. 2002).

However, postmortem studies have identified changes in the density and composition of glutamate receptors in the prefrontal cortex, thalamus and temporal lobe areas, with decreased activation during performance tests in schizophrenic patients. Chronic administration of phencyclidine reduces dopamine turnover in the frontal cortex and increases the release of dopamine in subcortical regions, particularly in the nucleus accumbens. These data demonstrate the interconnection of the dopaminergic and glutamatergic systems, showing that they are complementary concepts in understanding the pathogenesis of schizophrenia (Goff and Coyle 2001).

Glutamate plays a major role in neuronal migration, neurite development, synaptogenesis, neuronal pruning and apoptosis. There are a variety of glutamate receptor subtypes that are genetically encoded, but its expression can be altered by environmental factors during brain development, creating a model of glutamatergic dysfunction due to the interaction of genetic and environmental risk factors in schizophrenia (Goff and Coyle 2001). In a more recent formulation, dopaminergic hypofunction in the prefrontal cortex would be responsible for negative symptoms, and a primary event in schizophrenia, leading to a secondary dopaminergic hyperfunction in the striatum and, consequently, positive symptoms (Stone et al. 2007).

The above findings support the results of this research because the concentrations of BDNF were affected only in the prefrontal cortex at 1 and 24 h the last injection of ketamine. Both groups received treatment with ketamine (vehicle+ketamine or omega + ketamine). Therefore, it can be stated that the cortex was the brain structure that was most affected in this animal model, and omega-3 partially prevented the response at 24 h after the

last injection. These data suggest that longer and higher doses of omega-3 supplementation could be a possible treatment that potentially ensures better quality of life in schizophrenic patients.

The role of the omega-3 PUFAs in the brain are not completely elucidated, but some evidence supports the hypothesis that PUFAs are essential for normal brain function, as a part of the cell membrane, by facilitating the synaptic plasticity and improving mitochondrial function, in addition to reducing the intracellular oxidative stress (Gomez-Pinilla 2008). These neuroprotection proprieties would be involved in the prevention of the onset of schizophrenia in prodromal patients (Amminger et al. 2010).

The present study tested the hypothesis that omega-3 polyunsaturated fatty acids prevent biochemical changes in animals subjected to the ketamine-induced model of schizophrenia. Omega-3 supplementation delayed the effects of ketamine on the levels of BDNF, a possible indicator of protection of this fatty acid. In contrast, this experimental design generated similar levels of interleukins, indicating that the supplement and the ketamine treatment had no effect on this parameter. These results can be due to the mechanism of action of ketamine on the immune system. These data reinforce the important role that a diet rich in polyunsaturated fatty acids play on preventing the development of diseases, raising the need for knowledge on the mechanisms by which omega -3 generates such effects.

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RESUMO

Novos estudos sugerem que os ácidos graxos poliinsaturados, como o ômega-3, podem reduzir os sintomas da esquizofrenia. O presente estudo avaliou o efeito preventivo do ômega-3 sobre os níveis de interleucinas (IL) e o fator neurotrófico derivado do cérebro (BDNF) em cérebros de ratos jovens submetidos a um modelo de esquizofrenia. O tratamento foi feito por 21 dias, iniciando no 30º dia de vida dos animais. Depois de 14 dias de tratamento com ômega-3 ou veículo, um tratamento concomitante com salina ou ketamina (25 mg/kg) foi iniciado e mantido até o último dia do experimento. No cortex pré-frontal dos ratos, os níveis de BDNF diminuíram em 1 h e 24 h depois da última administração de ketamina enquanto que o grupo administrado com ketamina e ômega-3 mostrou uma diminuição nos níveis de BDNF somente após as 24 h. Em contraste, ambas as intervenções induziram respostas similares quanto aos níveis de IL-1β e IL6. Esses achados sugerem que a similaridade nos níveis de IL-1β e IL6 em nossos grupos de experimento é devida ao mecanismo de ação da ketamina sobre o sistema imune. Mais estudos são necessários para explicar essa patologia. Em conclusão, de acordo com estudos prévios e considerando o atual estudo, nós sugerimos que o ômega-3 tem um papel profilático no desenvolvimento de sintomas associados à esquizofrenia.

Palavras-chave: omega-3, ketamina, esquizofrenia, neurotrofinas, interleucinas.

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