Effects of omega-3 dietary supplement in prevention of positive, negative and cognitive symptoms: A study in adolescent rats with ketamine-induced model of schizophrenia

Clarissa S. Gama a,b,⁎, Lara Canever c, Bruna Panizzitti a,b, Carolina Gubert a,d, Laura Stertz a,d, Raffael Massuda a,b, Mariana Pedrini a,b, David F. de Lucena e,f, Renata D. Luca c, Daiane B. Fraga c, Alexandra S. Heylmann c, Pedro F. Deroza c, Alexandra I. Zugno c

a Laboratory of Molecular Psychiatry, INCT for Translational Medicine, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
b Programa de Pós-Graduação em Medicina: Psiquiatria, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
c Laboratório de Neurociências, Faculdade de Medicina Christus, Fortaleza, Ceará, Brazil
d Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
e Faculdade de Medicina Cristus, Fortaleza, Ceará, Brazil
f Laboratório de Neurofarmacologia, Departamento de Fisiologia e Farmacologia da Universidade Federal do Ceará, Fortaleza, Brazil

⁎ E-mail address: clarissagama@gmail.com (C.S. Gama).

Abstract

Omega-3 has shown efficacy to prevent schizophrenia conversion in ultra-high risk population. We evaluated the efficacy of omega-3 in preventing ketamine-induced effects in an animal model of schizophrenia and its effect on brain-derived neurotrophic factor (BDNF). Omega-3 or vehicle was administered in Wistar male rats, both groups at the 30th day of life for 15 days. Each group was split in two to receive along the following 7 days ketamine or saline. Locomotor and exploratory activities, memory test and social interaction between pairs were evaluated at the 52nd day of life. Prefrontal-cortex, hippocampus and striatum tissues were extracted right after behavioral tasks for mRNA BDNF expression analysis. Bloods for serum BDNF were withdrawn 24 h after the end of behavioral tasks. Locomotive was increased in ketamine-treated group compared to control, omega-3 and ketamine plus omega-3 groups. Ketamine group had fewer contacts and interaction compared to other groups. Working memory and short and long-term memories were significantly impaired in ketamine, ketamine plus omega-3 group compared to others. Serum BDNF levels were significantly higher in ketamine plus omega-3 group. There was no difference between groups in prefrontal-cortex, hippocampus and striatum for mRNA BDNF expression. Administration of omega-3 in adolescent rats prevents positive, negative and cognitive symptoms in a ketamine animal model of schizophrenia. Whether these findings are consequence of BDNF increase it is unclear. However, this study gives compelling evidence for larger clinical trials to confirm the use of omega-3 to prevent schizophrenia and for studies to reinforce the beneficial role of omega-3 in brain protection.

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1. Introduction

Schizophrenia (SZ) is a highly debilitating neurodevelopmental disorder that strikes at a critical period of a young person's life (Kaur and Cadenne, 2010). Early diagnosis and prevention has been one of the targets to improve long-term outcomes (Amminger et al., 2011a). In this context, it has been identified as a syndrome conferring ultra-high risk (UHR) for psychosis (Loewy et al., 2011, 2012). While there is a lower risk in UHR population for transition to psychosis in recent studies (Loewy et al., 2012) compared with initial studies (Phillips et al., 2000; Yung et al., 2004) most recent findings still show a rate that is substantially higher than the incidence rate of psychosis among transition age youth in the general population (Cannon et al., 2007; Cannon et al., 2008). It is possible that treatments that are not normally used in patients with psychotic disorders may prove effective when applied at this stage (Berger et al., 2012). Moreover, specific interventions designed to treat young persons who are identified as being at UHR of psychosis, might be associated with some cost savings compared with non-specific interventions (Phillips et al., 2009).

Currently evidence for specific intervention strategies for UHR population is moderate and requires replication with larger samples (Yung and Nelson, 2011). A meta-analysis has identified seven reports detailing results from five independent randomized clinical trial studies. The current results do not allow recommendation for any specific treatment...
A 12-week intervention double-blinded clinical trial to evaluate the use of omega-3 polysaturated fatty acids (PUFAs) in individuals at UHR for psychosis has shown that long-chain omega-3 PUFAs reduce the risk of progression to psychotic disorder and may offer a safe and efficacious strategy for indicated prevention (Amminger et al., 2010). A recent meta-analysis shows that omega-3 PUFAs had advantage over placebo (n = 76, 1 RCT, RR transition to psychosis 0.13 CI 0.02 to 1.0, NNT 6 CI 5 to 96) (Marshall and Rathbone, 2011).

The membrane phospholipidic hypothesis of SZ and other psychiatric disorders, and neurodegenerative diseases could result from abnormalities in the structure of the neuronal membrane phospholipid (Horrobin, 1998; Peet, 2008). The PUFAs, characterized as major structural components of cell membrane, are decreased in patients with SZ (Assies et al., 2001; Khan et al., 2002; Yao, 2003; Reddy et al., 2004; Peet, 2008). Recent evidence suggests that decreased levels of n-6 fatty acid, a monounsaturated omega-9 fatty acid important in the biosynthesis of myelin, correlate with prodromal symptoms, and predict conversion to psychosis in young people at high clinical risk for psychosis (Amminger et al., 2011b). There is also evidence that both arachidonic acid (omega-6) (Reddy et al., 2004) and n-3 fatty acid (omega-3) (Assies et al., 2001) may be reduced in individuals with SZ. It has been shown that these individuals present a reduction in long chain fatty-acids, especially eicosapentaenoic acid (omega-3), which may represent a lipid metabolism dysfunction involved in the etiology of the disorder (Horrobin et al., 1994; Skosnik and Yao, 2003; Amminger et al., 2010).

Recent studies highlight the possibility of omega-3 PUFAs to interact with the dopamine and serotonin system, since both are associated with the pathophysiology of SZ by modulating the receptor coupled to free arachidonic acid (Berger et al., 2008; Amminger et al., 2010; Amminger and McGorry, 2012). The eicosapentaenoic acid also seems to increase glutathione in the temporal lobe in first episode of psychosis patients with SZ. These findings indicate that glutathione may be impaired in SZ, thereby protecting neurons from excitotoxicity and oxidative stress, characteristic in patients with this disorder (Berger et al., 2008; Gama et al., 2008A; Kunz et al., 2011; Pedrini et al., 2012).

There is an increasing recognition that the pathophysiology of SZ could be the result of deregulation of synaptic plasticity with downstream alterations of neurotrophins (Gratocost, 2007). Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin in the central nervous system (CNS), and performs many biological functions such as neural survival, differentiation, and plasticity (Gama et al., 2007; Qian et al., 2007). Decreased serum BDNF levels have been reported in first-episode of SZ (Palomino et al., 2006). Omega-3 PUFAs could activate metabolic pathways to increase the BDNF levels (Balanza-Martinez et al., 2011).

Based on previous clinical findings (Amminger et al., 2010), omega-3 may be promising to be further tested in individuals at risk for psychosis. A better understanding of its mechanism of action in preclinical studies may point to relevant targets in UHR population and to the proposal of novel potential interventions. To that end, we evaluated the efficacy of omega-3 in preventing the effects of ketamine on behavior, BDNF serum levels and BDNF expression in an animal model of SZ.

2. Methods

Behavioral experiments were performed at the Laboratory of Neuroscience, Universidade do Extremo Sul Catarinense (UNESC), Brazil. The analysis of BDNF serum levels and BDNF expression was made in partnership in the Laboratory of Molecular Psychiatry, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil.

All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of Ethics Committee from UNESC.

2.1. Animals and treatments

We used young male Wistar rats weighing between 80 g and 150 g, obtained from Central Animal house of UNESC. The animals were kept in climate-controlled (22 °C) with light-dark cycle of 12 h; water and food were provided ad libitum throughout the treatment. The study used a total of 192 animals, which were divided randomly into four groups of 12 rats. 1) Omega-3 plus saline (called omega-3 group), 2) Omega-3 plus ketamine (called omega-3 plus ketamine group), 3) Vehicle plus saline (called control group), and 4) Vehicle plus ketamine (called ketamine group). The reason for four groups of comparison is the oily nature of omega-3 and the aqueous nature of ketamine. In this kind of pharmacological experiment, for each active substance there is a need of an inert substance similar in nature to be placebo.

The omega-3 PUFAs were administered once daily at a dose of 0.8 g/kg/bodyweight by gavage method. The vehicle was an inert oil with no impact on omega-3 fatty acid metabolism, it was chosen as placebo. This was also administered at the same concentration and by the same method as the omega-3 PUFAs.

Treatment with omega-3 or vehicle started in young animals at the 30th day of life with a total period of 15 days. After this period, the animals received ketamine or saline associated with the omega-3 or vehicle for over seven days, completing a total of 22 days of intervention. The fish oil capsules contained 1200 mg of oil that was rich in omega-3, composed by EPA (18%) and DHA (12%).

The animals were injected with ketamine (CU Chemie Uetikon, Germany) as an animal model of SZ, at a dose of 25 mg/kg, intraperitoneally (i.p.), prepared in saline at a volume of 1 mL/100 g (Becker and Grecksh, 2004; Imre et al., 2006; Tomiya et al., 2006). The administration of 25 mg/kg was used to mimic some psychotic symptoms such as blunted affect and hyperlocomotion (Hunt et al., 2006).

After the behavioral tests, rats were killed by guillotine decapitation and the brain structures (striatum, hippocampus and prefrontal cortex) were carefully removed for subsequent biochemical evaluations. For the BDNF serum measurement, rats were killed 24 h after the behavioral tests.

All procedures for disposal of animals followed the standards of the RDC No. 306/2004 ANVISA (National Agency for Sanitary Vigilance).

2.2. Behavioral assessment tools

2.2.1. Locomotor activity

We used the open-field task to assess locomotor activity. The task (n = 12) was assessed 30 min after the last injection of ketamine, using Activity Monitor to quantify behavioral changes induced by ketamine in animals and possibly prevented by omega-3. This activity was performed in a monitor 40×60 cm in size surrounded by acrylic walls approximately 50 cm tall. This monitor is surrounded by six parallel bars, each bar presented 16 infrared sensors that detect the exact position of the mouse movement rating, then the detailed behavior of the animal. The animals were evaluated for 15 min (de Oliveira et al., 2009).

The information detected by the sensors was transmitted to a computer using the software Open Source Interbase Version 6.01 (Activity Monitor — Insight Lab Equipment, Ribeirão Preto — SP, Brazil) for further statistical analysis.

2.2.2. Inhibitory avoidance

Assessment of inhibitory avoidance (n = 12) was initiated 24 h after the last injection of ketamine. Avoidance consists of a perspex...
box in which the floor is made up of parallel metal bars. A platform is placed at the left wall of the device (Quevedo et al., 1997; Roesler et al., 2003).

In the training session, the animals were placed on the platform to measure the time it takes for the animal down with four legs of this site (latency). Immediately after getting off the platform, the animal received a shock of 0.4 mA for 2 s.

In the test session, the animal was again placed on the platform and the time it took to fall (latency) was measured, but no shock was administered. Latency is characterized by classical parameter memory retention. The intervals between the training and test were measured immediately after training to assess working memory, 1.5 h after training to measure short-term memory (Izquierdo et al., 1998; Bevilaqua et al., 2003) and 24 h after to evaluate long-term memory (Bevilaqua et al., 2003; de Lima et al., 2005).

2.2.3. Social interaction
Impaired social interaction is a characteristic behavior of animal models of autism spectrum disorders and SZ (Mohn et al., 1999; Schneidert and Przewlocki, 2005; Dicicco-Bloom et al., 2006; Moldin et al., 2006).

The animals (n = 12) were tested in ambient light/dark and unfamiliar conditions, in an open field apparatus. On the day of the experiment the animals are isolated socially in a plastic box measuring 43 × 28 × 15 cm material for 3 h before the experiment.

The task consisted of placing two animals of the same group randomized into cages for 15 min. The social behavior was assessed for a pair of animals, so behavior of individual animals was not analyzed (Schneidert and Przewlocki, 2005). The latency to the first interaction, the number of social contacts and total time were measured (Niesink and Van Ree, 1989; Schneidert and Przewlocki, 2005).

2.3. Biochemical analysis

2.3.1. Preparation of samples
After death by decapitation, striatum, prefrontal cortex and hippocampus were removed. One hemisphere was frozen at −80 °C for subsequent biochemical analysis. The other hemisphere was stored in RNAlater® (Sigma-Aldrich, USA) for RNA extraction and further gene expression analysis. Before the procedures rat brain tissues were homogenized in phosphate buffer solution, PBS (Laborclin, Brazil) with protease inhibitor cocktail (Sigma-Aldrich, USA) and centrifuged at 5000 rpm for 5 min. The supernatant was separated for the assays.

2.3.2. BDNF measurement
Microtiter plates (96-well flat-bottom) were coated overnight at 4 °C with the samples diluted 1:3 in sample diluent and standard curve ranged from 7.8 to 500 pg/mL of BDNF. BDNF serum levels were measured by a commercial BDNF sandwich-ELISA kit, according to the manufacturer’s instructions (Millipore, USA & Canada). Total protein was measured by Coomassie Blue method using bovine serum albumin as a standard.

2.4. Gene expression of BDNF mRNA

2.4.1. Extraction of RNA and cDNA synthesis
Total RNA was isolated from brain structures submerged in RNAlater® (Sigma-Aldrich, USA) using TRI Reagent® (Sigma-Aldrich, USA) according to manufacturer’s instructions. The quantity and purity were evaluated by spectrophotometry (NanoDrop™ 1000, Thermo Scientific, USA) and the total RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), according to manufacturer’s instructions. Subsequently, the cDNA was maintained at −20 °C until use for PCR amplification.

2.4.2. Real-time Quantitative PCR (qPCR)
The expression of BDNF was measured by qPCR using a TaqMan assay FAM/MGB (Applied Biosystems, Rn01484928_m1 ID test). Expression values were normalized by the expression of endogenous beta-actin and GAPD control using an assay for endogenous control TaqMan VIC/MGB (Applied Biosystems, 4352340E and 4352335E). The reactions were performed on equipment 7500 Real Time PCR System (Applied Biosystems, USA). Program cycles were 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 15 s, 95 °C and 1 min at 60 °C. All reactions were carried out in triplicate. Relative expression levels were then determined by the method ΔΔCT.

2.5. Statistical analysis
Data from the expression of BDNF are reported as median and interquartile range. Comparisons among groups were performed using Kruskal–Wallis test either for BDNF expression or for behavioral tasks. Data from the biochemical analyses are reported as ANOVA followed by Tukey post hoc test and expressed as mean ± S.D. In all comparisons, p < 0.05 indicated statistical significance.

3. Results

3.1. Behavioral tests
At 5, 10 and 15 minutes there was an increase in locomotor activity in the ketamine-treated group (p = 0.0004). A significant prevention of positive symptoms in this ketamine model could be seen with omega-3 treatment at 10 (p = 0.0033) and 15 (p = 0.042) minutes (Fig. 1).

Ketamine also induces severe social deficits, as seen in the social interaction in these animals; omega-3 was able to prevent the effects usually observed with ketamine. Animals treated with ketamine showed no difference in onset latency of the test (p = 0.333); however they had fewer contacts with each other (p = 0.0001), as well as lower total time of interaction (when compared to control, omega-3 and ketamine plus omega-3 groups (p = 0.0001) (Fig. 2)).

In the ketamine plus omega-3, omega-3 and control groups, working memory, as well as the short and long-term memories were preserved compared to ketamine group (p = 0.0015), confirming the preventive role of omega-3 in cognitive deficits (Fig. 3).
3.2. Biochemical measurements

BDNF levels were significantly higher in ketamine plus omega-3 group compared to control, omega-3 and ketamine groups \((p = 0.017)\) (Fig. 4).

There was no significant difference between groups in prefrontal cortex \((p = 0.668)\), hippocampus \((0.063)\) and striatum \((p = 0.580)\) for the mRNA expression of BDNF (Table 1).

4. Discussion

To our knowledge, this is the first study to show the use of omega-3 for positive, negative and cognitive symptom prevention in a ketamine-induced animal model of SZ.

The results suggest that serum BDNF levels were significantly higher in ketamine plus omega-3 group. However, there was no difference between groups in prefrontal-cortex, hippocampus and striatum for mRNA BDNF expression. The results also suggest that the administration of omega-3 in adolescent’s rats prevents positive, negative and cognitive symptoms in a ketamine animal model of schizophrenia.

Agents that block the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, such as ketamine, induce a SZ-like psychosis in adult humans (Hunt et al., 2006; Adler et al., 1999; Krystal et al., 1994) and injure or kill neurons in several cortico-limbic regions of the adult rat brain (Farber et al., 1995). Vulnerability of rats at various ages to the neurotoxic effects of a powerful NMDA antagonist was found to be age dependent, having onset at approximately puberty (45 days of age) and becoming maximal in early adulthood (Farber et al., 1995). This age-dependency profile (onset of susceptibility in late adolescence) in the rat is similar to that for ketamine-induced psychosis or SZ in humans (Farber et al., 1995). Since Ketamine has these properties and can cause oxidative stress on animal model (de Oliveira et al., 2009; de Oliveira et al., 2011), this drug was elected by us to induce SZ-like condition in this study.

Increased BDNF levels by omega-3 have been previously described (Wu et al., 2004). It is also known that ketamine increases BDNF levels (Autry et al., 2011; Réus et al., 2011; Ricci et al., 2011). Omega-3 PUFAs would be involved in the activation of several metabolic pathways to increase the BDNF levels (Balanza-Martinez et al., 2011). In our study, BDNF levels were significantly higher in ketamine plus omega-3 group compared to control, omega-3 and ketamine groups \((p = 0.017)\), showing that omega-3 further increased these levels. Contrasting to the literature (Wu et al., 2004), omega-3 alone did not increase BDNF levels in our study.

This study found differences in BDNF serum levels, but no differences in brain structures for mRNA BDNF expression between groups. The lack of difference between groups on BDNF expression in prefrontal cortex, hippocampus and striatum would be explained by the mismatch
between the BDNF protein distribution in serum and the cellular BDNF mRNA expression in tissues (Tropea et al., 2001). In addition, it is important to consider that in our study, tissue was obtained right after the end of behavioral tasks and serum 24 h after.

In spite of oxidative stress analysis not being the objective of this study, the involvement of the oxidative stress in the pathophysiology of SZ has been widely described (Gama et al., 2006, 2008B; Kunz et al., 2008) and it is present in the early and late phases of the disorder (Pedriini et al., 2012). Moreover, BDNF serum levels seem to be inversely related to some oxidative stress product markers (Kapczinski et al., 2008). The oxidative cell damage (i.e., DNA breaks, protein inactivation, altered gene expression, loss of membrane lipid-bound essential polyunsaturated fatty acids and often apoptosis) contributes to abnormal neural growth and differentiation (Mahadik et al., 2006). The role of the omega-3 PUFAs in the brain is not completely elucidated, but some evidences support that PUFAs are essential for the normal brain function, as part of the cell membrane, facilitating the synaptic plasticity and improving mitochondrial function, in addition to reducing the intracellular oxidative stress (Gomez-Pinilla, 2008). These brain protection proprieties would be involved in the prevention of the onset of SZ in prodromal patients (Amminger et al., 2010).

The current study shows that the administration of omega-3 PUFs prevents positive, negative and cognitive symptoms in a ketamine animal model of SZ. These results are in line with Amminger et al.’s (2010) clinical trial of omega-3 in UHR population to develop SZ. Besides that, a combination of antioxidants and omega-3 PUFAs, particularly in the early stages of illness, when the brain has a high degree of neuroplasticity, potentially may be even more effective for long-term improved clinical outcome of SZ (Mahadik et al., 2006). We focused our experiments on BDNF expression and levels. Other mechanisms like omega-3 augmentation altering glutathione availability and modulating the glutamate/glutamine cycle in early psychosis, with some of the metabolic brain changes being correlated with negative symptom improvement were reported (Berger et al., 2008).

The biochemical mechanisms of neuroprotection to explain these findings are unclear and deserve further investigation. Nevertheless, this study gives compelling evidence for larger clinical trials to confirm the use of omega-3 PUFAs to prevent SZ and for studies to reinforce the beneficial role of the PUFAs in brain protection of UHR individuals for psychosis.

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### Conflict of interest

The authors have declared no conflict of interest in this matter.

### Acknowledgements

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### References


### Table 1

<table>
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<th>Striatum</th>
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<td>Interquartile amplitude</td>
<td>Median</td>
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<tr>
<td>p value(^a)</td>
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Values are expressed as median and interquartile amplitude.

\(^a\) Kruskal–Wallis test.

![Fig. 4](image-url) Effects of omega-3 supplementation and ketamine treatment on brain-derived neurotrophic factor (BDNF) levels. Bars represent means and S.D. BDNF levels were significantly higher in ketamine plus omega-3 group compared to control, omega-3 and ketamine groups, p = 0.017 as indicated by *.


Quevedo, J., Vianna, M., Zanatta, M.S., et al., 1997. Involvement of mechanisms dependent on NMDA receptors, nitric oxide and protein kinase A in the hippocampus but not in the caudate nucleus in memory. Behav. Pharmacol. 8, 713–717.


