The Antipsychotic Effects of Omega-3 Fatty Acids in Rats

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Abstract: *Background:* In humans, omega-3 fatty acids are necessary for cell membranes, brain function and nerve transmission continuation. When animals are exposed to a new environment—or as a result of an apomorphine application that creates an agonistic effect on D1 and D2 receptors—they display behavioral reactions like rearing and stereotypy. This study aims to reveal the possible antipsychotic and oxidative effects of omega-3 fatty acids by comparing with chlorpromazine, a conventional antipsychotic drug, through evaluating the novelty-induced rearing and apomorphine-induced stereotypic behaviors, as well as malondialdehyde and glutathione levels in rats. *Methods:* Twenty-eight, adult, male, Wistar rats were used in the study. Briefly, 4 groups of rats (n = 7) were administered docosahexaenoic acid (DHA) + eicosapentaenoic acid (EPA) (300 mg/kg; DHA: 120 mg/kg + EPA: 180 mg/kg intraperitoneally [IP]), DHA + EPA (150 mg/kg; DHA: 60 mg/kg + EPA: 90 mg/kg IP), chlorpromazine (1 mg/kg, IP) and isotonic saline (1 mL/kg, IP). One hour later, apomorphine (2 mg/kg, subcutaneously) was administered to each rat. After the apomorphine administration, rats were observed for stereotypic behavior. *Results:* This study shows that omega-3 fatty acids, "similar to antipsychotics," reversed the psychotic like effects, increase of oxidants and decrease of antioxidants that are composed experimentally in rats. *Conclusions:* The application of omega-3 fatty acids has antipsychotic effects and causes an oxidative imbalance. This study adds new evidence to the current literature regarding the possible antipsychotic effects of omega-3 fatty acids.

Key Indexing Terms: Omega-3 fatty acids; Antipsychotic effect; Oxidative stress. **[Am J Med Sci 2015;350(3):212–217.]**

A growing amount of evidence suggests that the main problem in schizophrenia is dopaminergic hyperactivation. Studies suggest that dopamine is effective in therapeutic interventions by inhibiting postsynaptic dopamine receptors.[1] Although the disease's etiology is still unknown, abnormal neuronal maturation, neuronal and glial cell migration, dendritic and axonal branching and truncation, programmed cell death or other reasons, such as stress, trauma, infection and substance use, are thought to be involved in the disease onset.[2–5] Oxidative stress results when excessive amounts of oxidants are produced and enzymatic and nonenzymatic antioxidant defense systems are insufficient. The failure of antioxidant mechanisms and membrane metabolism disorders is believed to play a role in neuronal destruction.[6] Although oxidative stress is not the main cause of schizophrenia, it has a great impact on the pathophysiology of schizophrenia.

Dopamine metabolism is one of the possible sources of oxidative stress forming reactive products.[7] Oxidative stress resulting from elevated dopamine levels can cause late-onset and permanent central nervous system damage by increasing the striatal glutamatergic neurotransmission.[7] It is believed that oxidative stress causes disease as a result of its toxic effects on carbohydrates, proteins, lipids and DNA metabolism.[8] The effects of oxidative products can lead to malfunctioning enzymes, neurotransmitters and receptor proteins and may cause a disruption in membrane integrity by reducing the fluidity and permeability of the cell membrane.[9] In accordance with the hypothesis of the membrane phospholipids in other psychiatric disorders and neurodegenerative diseases, structural abnormalities of neuronal membrane phospholipids can be an important factor for schizophrenia etiology.[10]

Studies on lipid peroxidation markers, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), showed that elevated levels of MDA were found in patients with negative symptoms, indicating greater oxidative damage in this patient group.[7] Elevated MDA levels have also been found in peripheral tissues of patients with schizophrenia.[11–13] Glutathione (GSH) depletion, a nonenzymatic antioxidant component, and GSH-related enzyme deficits are also extensively documented in schizophrenia.[14–16] Ballesteros et al[17] reported significantly lower GSH and higher glutathione disulfide (GSSG) levels in the blood of schizophrenia individuals.

In mammals, omega-3 polyunsaturated fatty acids (n-3 PUFA) are required for cell membranes, brain function and transmission maintenance of nerve impulses. PUFAs are found in the brain and blood cells. Docosahexaenoic acid (DHA), a PUFA, regulates cell transport and synaptic functions.[18]

The main structural components of membrane phospholipids are PUFAs, which are not *de novo*—synthesized from fatty acids in the body.[10] The presence of PUFAs is known to increase membrane fluidity. Consequently, in case of a PUFA deficiency, membranes become more rigid and result in changes in conformation and function of proteins, receptors and ion channels.[19,20] While PUFAs are not antioxidants, they display antioxidant effects similar to classic antioxidants such as vitamin E.[21] Also, it is well known that there is an oxidative imbalance in schizophrenia.[22]

Clarifying the mechanisms of PUFA deficits in schizophrenia may provide important insight into the underlying pathophysiology. In rats, the addition of n-3 to ketamine-induced schizophrenia models was shown to lower positive, negative and cognitive symptoms.[22] In another study, addition of n-3 inhibited the startle reflex, reduced lipid damage in the hippocampus and striatum and reduced protein destruction in the prefrontal cortex. Additionally, n-3 may play a prophylactic role against symptoms associated with schizophrenia.[23] One study showed that animals supplemented with trans fats, which contain n-3 fatty acids, from an early age presented stronger behavioral and biochemical amphetamine-induced responses.[24]

There are limited preclinical studies about the antipsychotic effect of n-3, and these have conflicting results. In the present study, it is hypothesized that n-3 will produce an antipsychotic effect in a rat model. To determine its efficacy in psychosis, MDA and GSH levels were compared with the effects

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of n-3 and chlorpromazine by evaluating the novelty-induced rearing and apomorphine-induced stereotypic behavior in rats.

MATERIALS AND METHODS

Animals

The experimental protocol performed in the study was approved by the Institutional Animal Care and Ethics Committee of the Gaziosmanpasa University. Twenty-eight adult male Wistar rats (age, 7–8 weeks old; weight, 220–240 g) were used in the study. All animals were kept under standard 12-hour light/dark cycles in a temperature-controlled (22 ± 2°C) environment with *ad libitum* access to rodent chow. All experimental procedures were performed during the light cycle.

Chemicals

All drugs were freshly prepared. Apomorphine hydrochloride (Sigma Chemical, Co, St Louis, MO) was dissolved in saline containing 0.1% ascorbic acid before experiments. DHA[25] and eicosapentaenoic acid (EPA) (Marincap; Kocak, Turkey) was prepared. Saline (0.9% NaCl) was used as a control solution. All solutions were administered intraperitoneally (IP).

Assessment of Novelty-Induced Rearing Behavior

Novelty-induced rearing behavior is used to assess the central excitatory locomotor behavior in rodents.[23] Four groups of rats (n = 7) were administered DHA + EPA (300 mg/kg; DHA: 120 mg/kg + EPA: 180 mg/kg IP),[26] DHA + EPA (150 mg/kg; DHA: 60 mg/kg + EPA: 90 mg/kg IP),[27] chlorpromazine (1 mg/kg; IP) or isotonic NaCl (1 mL/kg, IP). One hour later, novelty-induced rearing behavior was assessed by placing the animals directly from their home cages into a transparent Plexiglas cage (45 × 25 × 25 cm). The rearing frequency (number of times the animal stood on its hind limbs, with its fore limbs against the walls of the observation box or free in the air) was recorded for 10 minutes. All rats were monitored individually by 2 observers who were blind to the study groups. The arena was cleaned with 5% alcohol to eliminate olfactory bias before beginning with a fresh animal.[28]

Apomorphine-Induced Stereotypic Behavior Test

Mesolimbic and nigrostriatal dopaminergic pathways play crucial roles in the mediation of locomotor activity and stereotypic behavior. Apomorphine-induced stereotypy is because of the stimulation of dopamine receptors and has been used as a convenient method for *in vivo* screening of dopamine agonists or antagonists and assessment of dopaminergic activity.[29,30] Four groups of rats (n = 7) were administered DHA + EPA (300 mg/kg; DHA: 120 mg/kg + EPA: 180 mg/kg IP), DHA + EPA (150 mg/kg; DHA: 60 mg/kg + EPA: 90 mg/kg IP), chlorpromazine (1 mg/kg, IP) and isotonic saline (1 mL/kg, IP). One hour later, apomorphine (2 mg/kg, subcutaneously) was administered to each rat. First, rats were placed into cylindrical metal cages (18 × 19 cm) containing vertical (1 cm apart) and horizontal (4.5 cm apart) metal bars (2 mm) with upper lids for a 10-minute orientation period. After apomorphine administration, the rats were immediately placed back into the metal cages and observed for stereotypic behavior. Signs of stereotypy, which include sniffing and gnawing, were observed and scored as follows: absence of stereotypy (0), occasional sniffing (1), occasional sniffing with occasional gnawing (2), frequent gnawing (3), intense continuous gnawing (4) and intense gnawing on the same spot (5). The stereotypic behavior was rated after each minute, and a mean of 15-minute periods was calculated and recorded.[31] Assessment of stereotypic behavior was performed by 2 observers blind to the study groups.

Measurement of Brain Lipid Peroxidation

Lipid peroxidation was determined in tissue samples by measuring MDA levels as thiobarbituric acid reactive substances (TBARS).[32] Trichloroacetic acid and TBARS reagents were added to the tissue samples, then mixed and incubated at 100°C for 60 minutes. After cooling on ice, the samples were centrifuged at 3000 repetitions per minute for 20 minutes, and the absorbance of the supernatant was read at 535 nm. MDA levels were calculated from the standard calibration curve using tetraethoxypropane and expressed as nanomoles/gram of protein.

Measurement of Brain Protein Levels

Total protein concentration in brain samples was determined according to Bradford's[33] method using bovine serum albumin as the standard.

Measurement of Tissue Glutathione Levels

GSH content in tissue samples was measured spectrophotometrically according to Ellman's[34] method. In this method, thiols interact with 5,5'-dithiobis (2-nitrobenzoic acid) and form a colored anion with a maximum peak at 412 nm. GSH levels were calculated from the standard calibration curve and expressed as micromoles/gram of protein.

Statistical Analysis

Statistical evaluation was performed by 1-way analysis of variance. A *post hoc* Bonferroni's test was used to identify differences between the experimental groups. MDA and GSH levels were evaluated between and within the groups by the Kruskal-Wallis variance analysis and the Mann-Whitney's *U* test where appropriate. Results are presented as mean ± standard error of the mean. A value of $P < 0.05$ was considered to be significant.

RESULTS

DHA + EPA (150 mg/kg), DHA + EPA (300 mg/kg) and chlorpromazine significantly decrease apomorphine-induced stereotypy scores compared to the control group ($P < 0.001$) (Figure 1). DHA + EPA (300 mg/kg) and chlorpromazine significantly decrease rearing behavior scores and brain MDA levels compared to the control group ($P < 0.001$) (Figures 2 and 3). DHA + EPA (150 mg/kg) significantly decrease rearing behavior scores and brain MDA levels compared to the control group ($P < 0.01$) (Figures 2 and 3). DHA + EPA (150 mg/kg) and chlorpromazine significantly increase brain GSH levels compared to the control group ($P < 0.05$) (Figure 4). DHA + EPA (300 mg/kg) significantly increase brain GSH levels compared to the control group ($P < 0.001$) (Figure 4).

DISCUSSION

This study demonstrates the beneficial effects of n-3 on rearing behavior and stereotypy, which are accepted as indicators of antipsychotic effects. Theoretically, antipsychotic effects are formed by means of antidopaminergic activity in certain regions of the central nervous system. Antipsychotics have various side effects that are poorly tolerated. Moreover, two thirds of schizophrenics do not respond optimally to antipsychotic drugs.[35] Therefore, clinical and nonclinical investigations focus on new drugs or supplements, which have fewer side effects.