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OMEGA-3 FATTY ACIDS HAVE ANTIDEPRESSANT ACTIVITY IN FORCED SWIMMING TEST IN WISTAR RATS

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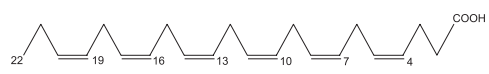
Abstract: Forced swimming test is used to induce a characteristic behavior of immobility in rats, which resembles depression in humans to some extent. We evaluated the effect of omega-3 fatty acids alone as well as compared it with the standard antidepressant therapy with fluoxetine in both acute and chronic studies. In both the studies, rats were divided into 4 groups and subjected to the following drug interventions – Group 1- control; Group 2- fluoxetine in dose of 10 mg/kg subcutaneously 23.5, 5 and 1 h before the test; Group 3- omega-3 fatty acids in dose of 500 mg/kg orally; Group 4- fluoxetine plus omega-3 fatty acids both. In acute study, omega-3 fatty acids were given in single dose 2 h prior to the test while in chronic study omega-3 fatty acids were given daily for a period of 28 days. All animals were subjected to a 15-min pretest followed 24 h later by a 5-min test. A time sampling method was used to score the behavioral activity in each group. The results revealed that in acute study, omega-3 fatty acids do not have any significant effect in forced swimming test. However, in chronic study, omega-3 fatty acids affect the immobility and swimming behavior significantly when compared with control ($p < 0.01$) without any significant effect on climbing behavior and the efficacy of combination of omega-3 fatty acids and fluoxetine is significantly more than that of fluoxetine alone in changing the behavioral activity of rats in forced swimming test. It leads to the conclusion that omega-3 fatty acids have antidepressant activity per se, and the combination of fluoxetine and omega-3 fatty acids has more antidepressant efficacy than fluoxetine alone in forced swimming test in Wistar rats.

Keywords: omega 3 fatty acids, depression, forced swimming test, fluoxetine, immobility, swimming.

Mental depression is an illness that can have lasting emotional and physical manifestations, such as feelings of worthlessness, helplessness, hopelessness, guilt or indecision, change in appetite, change in sleep habits, loss of concentration, loss of energy, loss of interest, loss of pleasure, agitation, mental and motor slowing, drug or alcohol abuse, and social withdrawal and solitariness (1). During the last 100 years, the age of onset of major depression has decreased and its overall incidence has increased remarkably (2). This increase cannot be explained merely by changes in attitude of health professionals or society, diagnostic criteria, reporting bias, or institutional or other artifacts (3, 4). Further, this increase in the incidence of depression can be correlated with the fact that the intake of omega-3 fatty acids has dramatically declined in our diet over last 100 years. According to the conclusion of an international panel of lipid experts published in the Journal of the American College of Nutrition, the ideal ratio of omega-3 to omega-6 EFAs is approximately 1:1 while our diet currently has omega-6 fatty acids grossly outnumbering omega-3 fatty acids by a ratio of 20:1. Further, various epidemiological studies suggest that there exists a significant negative correlation between worldwide fish consumption and prevalence of depression (5, 6).

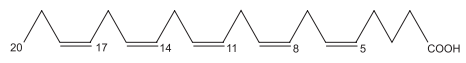
Omega-3 fatty acids are long-chain polyunsaturated fatty acids (18-22 carbon atoms in chain length) with the first of many double bonds beginning with the third carbon atom (when counting from the methyl end of the fatty acid molecule). The molecular structures of omega-3 fatty acids (DHA and EPA) are as follows:

1. DHA – $C_{22}H_{32}O_2$



Docosahexa-4,7,10,13,16,19-enoic acid

2. EPA – $C_{20}H_{30}O_2$



Eicosapenta-5, 8,11,14,17-enoic acid

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Long chain polyunsaturated fatty acids cannot be formed *de novo* but can be synthesized from the essential fatty acids: linoleic acid (LA) and alpha-linolenic acid (ALA). Linoleic acid (LA) is the parent compound of the omega-6 family of fatty acids, as is alpha-linolenic acid of the omega-3 family. These parent fatty acids are desaturated and lengthened progressively by microsomal enzyme systems to form highly unsaturated, long-chained arachidonic acid and docosahexaenoic acid. Members of the omega-6 and omega-3 families are not interconvertible and they also compete for the same enzyme systems. Dietary fish and fish oil supplements are a direct source of eicosapentaenoic acid and docosahexaenoic acid.

The forced swimming test was proposed as a model to test for antidepressant activity by Porsolt et al. in 1977 (7-13). It was suggested that rats when forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. The development of immobility is usually facilitated by a pretest of 15 min followed by a test period after 24 h.

The forced swimming test is considered a reliable and sensitive method to test the effects of all the major classes of antidepressants. The test originally described by Porsolt described only one method for scoring antidepressant efficacy that is immobility, which was considered insufficient to describe any active behaviors that are produced by antidepressant drugs in the forced swimming test. It was subsequently shown by Detke et al. (14) that it is more appropriate to score the active behaviors of rat that are swimming and climbing along with immobility, as few antidepressants produce a characteristic set of active behaviors during the forced swimming test and the traditional scoring method described by Porsolt, which focuses only on immobility, may fail to detect the response.

Our study is an attempt to evaluate the role of omega-3 fatty acids in experimental models of depression. The effect of omega-3 fatty acids in depression was compared with the standard antidepressant treatment with fluoxetine, which is a selective serotonin reuptake inhibitor (SSRI) and one of the first choice drugs in depression (15).

MATERIALS AND METHODS

Animals – a total of 128 male Wistar rats of weights in the range of 150-250 g were used. Animals were housed under standard conditions of temperature and humidity with a 12:12 light:dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The animal handling was performed according to the Good laboratory practice (GLP) guidelines.

Drugs – fluoxetine hydrochloride was obtained from Cadila Pharma, Ahmedabad, India and omega-3 fatty acids were obtained from Dr. Reddy's Laboratories Ltd., Hyderabad, India.

The effect of omega-3 fatty acids was evaluated alone and in combination with fluoxetine in both acute and chronic studies. Omega-3 fatty acids were administered in pure form to the rats without the use of any vehicle. Comparisons were made in forced swimming test. between the control group for omega-3 fatty acids that is rats fed on standard diet, with control group for fluoxetine that is rats administered with distilled water subcutaneously. No significant difference was found between the two controls in forced swimming test and hence rats administered with distilled water subcutaneously were used as control for both fluoxetine as well as omega-3 fatty acids group.

Acute study

The rats were divided into four groups of 16 each and the drugs were administered to rats as follows:

Group 1 (control) – distilled water 1 mL/kg subcutaneously 23.5, 5 and 1 h before the test.

Group 2 (fluoxetine) – fluoxetine hydrochloride dissolved in distilled water in a dose of 10 mg/kg subcutaneously 23.5, 5 and 1 h before the test (14).

Group 3 (omega-3) – omega-3 fatty acids in a dose of 500 mg/kg orally 2 h before the test (16).

Group 4 (fluoxetine plus omega-3) – rats were administered with omega-3 fatty acids in a dose of 500 mg/kg orally 2 h before the test and fluoxetine hydrochloride dissolved in distilled water in a dose of 10 mg/kg subcutaneously 23.5, 5 and 1 h before the test.

Chronic study

The protocol of drug administration was the same as of acute study except that in chronic study omega-3 fatty acids were given in a dose of 500 mg/kg orally for 28 days prior to the test both alone, as well as in combination with fluoxetine. The last dose of omega-3 fatty acids was administered 2 h prior to the test.

The forced swimming test

Naive rats are individually forced to swim inside a vertical Plexiglas cylinder (height 40 cm; diameter 20 cm; containing 30 cm of water main-

Table 1. Comparison of counts of immobility, swimming and climbing behaviors for different drug treatments in acute study.

GROUP		Counts of immobility (Mean ± SEM)	Counts of swimming (Mean ± SEM)	Counts of climbing (Mean ± SEM)
Control		29.62 ± 1.11	18.01 ± 1.15	12.37 ± 0.61
Fluoxetine		22.18 ± 1.03[a]	24.37 ± 1.18[a]	13.43 ± 0.59
Omega-3 fatty acids		28.50 ± 1.12	18.93 ± 1.02	12.56 ± 0.72
Fluoxetine + omega-3 fatty acids		21.81 ± 1.02[a]	24.68 ± 1.20[a]	13.50 ± 0.71
One-way ANOVA	F	14.60	9.51	0.77
	df	3,60	3,60	3,60
	p	< 0.001	< 0.001	0.51

n = 16 in each group. [a] $p < 0.01$ when compared with control.

Table 2. Comparison of counts of immobility, swimming and climbing behaviors for different drug treatments in chronic study.

GROUP		Counts of immobility (Mean ± SEM)	Counts of swimming (Mean ± SEM)	Counts of climbing (Mean ± SEM)
Control		30.56 ± 1.04	17.87 ± 0.98	11.56 ± 0.59
Fluoxetine		21.25 ± 1.03[a]	25.50 ± 1.05[a]	13.37 ± 0.52
Omega-3 fatty acids		24.18 ± 1.09[a]	22.81 ± 1.16[a]	13.06 ± 0.79
Fluoxetine + omega-3 fatty acids		16.12 ± 0.92[a,b]	30.12 ± 0.99[a,c]	13.75 ± 0.69
One-way ANOVA	F	34.541	23.705	2.103
	df	3,60	3,60	3,60
	p	< 0.001	< 0.001	0.1

n = 16 in each group. [a] $p < 0.01$ when compared with control, [b] $p < 0.01$ when compared with fluoxetine, [c] $p < 0.05$ when compared with fluoxetine

tained at 25°C). Rats are exposed to a 15-min pretest swim period and followed the next day with a 5-min test swim session. Both the swim sessions are conducted between 12.00-18.00 h. After each swim session, the rats are removed from water, dried with towels and placed in warm enclosure for 20 min and then returned to their home cages. The cylinders were emptied and washed thoroughly after testing for each rat. In the pretest forced swimming session of 15 min, rats initially are highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min activity begins to subside producing a characteristic behavior called immobility, in which the rat makes only those movements necessary to keep its head above water. The immobility increased when the rats were subjected to 5-min test sessions of forced swimming 24 h later. This "facilitated immobility" is known to be attenuated by antidepressant drug intervention. In the test session, a time-sampling technique was employed to score several behaviors during a single viewing. The rat's behavior was judged in blocks of 5 s and at the end of each 5-s period, the rat's behavior was judged as one of the following three behaviors: 1) immobility – when the rat remained floating in the water without struggling, and making only those movements necessary to keep its head above water 2) swimming – a rat was judged to be swimming if it was making active swimming movements, more than necessary to merely maintain its head above water, e.g. moving around in the cylinder; 3) climbing – a rat was judged to be climbing when it was making active movements with its forepaws in and out of the water, usually directed against the walls.

The swim tests were videotaped from the sides of cylinder using a Sony color camera with recorder and the behavioral scoring was done by a rater who was blinded to the treatment condition.

Statistical analysis

The means of behavioral scores were analyzed by one-way analysis of variance (ANOVA) for each behavior that is immobility, swimming and climbing, further followed by Tukey's multiple comparisons test, in both acute and chronic studies. $p < 0.05$ was considered as statistically significant.

RESULTS

In acute study, it was found that fluoxetine affected scores of immobility, swimming and climbing behaviors significantly ($p < 0.01$) while omega-3 fatty acids did not affect these behavioral scores significantly when compared with control. The combination of omega-3 fatty acids and fluoxetine also do not show any significant difference in behavioral scores when compared with fluoxetine.

In chronic study, it was found that both fluoxetine and omega-3 fatty acids as well as their combination affected the score of immobility significantly [$F(3,60) = 34.541$, $p < 0.001$]. The same drug interventions also affected the number of occurrences of swimming behavior significantly [$F(3,60) = 23.705$, $p < 0.001$]. However, these drug interventions did not affect the climbing behavior significantly [$F(3,60) = 2.103$, $p = 0.1$]. Further, the Tukey's multiple comparisons test showed that both the fluoxetine and omega-3 fatty acids significantly decrease the total counts of immobility ($p < 0.01$) when compared with control, and their combination that is fluoxetine + omega-3 fatty acids decreased the total score of immobility when compared with fluoxetine alone ($p < 0.01$). Both fluoxetine and omega-3 fatty acids increased the score of swimming behavior significantly when compared with control ($p < 0.01$), and their combination that is fluoxetine + omega-3 fatty acids increased significantly the score of swimming when compared with fluoxetine alone ($p < 0.05$).

DISCUSSION

It is evident from our study that fluoxetine, omega-3 fatty acids and their combination significantly affected the immobility and swimming behaviors without significantly affecting the climbing behavior. Our findings are in accordance with the study of Detke et al. (14) that showed that the climbing behavior was specifically affected by norepinephrine reuptake inhibitors, while the other classes of antidepressants affected the behaviors of immobility and swimming. The present study thus shows that the omega-3 fatty acids have antidepressant activity per se in depression, and the combination of omega-3 fatty acids and fluoxetine have significantly more antidepressant efficacy than the standard antidepressant therapy that is fluoxetine alone. The exact mechanism underlying the antidepressant action of omega-3 fatty acids is not clear but previous studies suggest the following possible mechanisms.

A growing body of research indicates that depression is associated with excessive production of proinflammatory cytokines. These cytokines, including interleukin-1 beta (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL–6), interferon-gamma (IF-g) and tumor necrosis factor-alpha (TNF-α), can have direct and indirect effects on the central nervous system**.** They lower the neurotransmitter precursor availability, activate the hypothalamic-pituitary axis, and alter the metabolism of neurotransmitters and neurotransmitter transporters. The elevations in IL-1β and TNF-α are associated with severity of depression (17). Psychological stress, infection, trauma, allergies, toxins, and various other factors can be responsible for a rise of these cytokines. Omega-3 fatty acids are well-documented inhibitors of proinflammatory cytokines, particularly TNF-α and IL-1 although the precise mechanism remains unclear (18). Kaneniwa and coworkers performed *in vitro* experiments on mouse mast cells and suggested that the omega-3 fatty acids induced suppression of prostaglandin E2 (PGE2), thromboxane A2 and histamine which are involved in anti-inflammatory effects and therefore, alleviation of depressive symptoms (19). In a similar pattern, Kuwamori et al. studied the effect of dietary omega-3/omega-6 fatty acids ratio on the total count, fatty acid composition, and histamine and leukotriene concentrations of mast cells in tunica mucosa bronchiorum of Type I allergic guinea pig and confirmed the similar findings (20).

Until recently, the notion that neurogenesis takes place in adult humans was not even considered. Now, however, researchers suggest that depression may inhibit neurogenesis in the hippocampus (21). This idea is supported by the finding that antidepressants can promote neurogenesis (22). Antidepressant treatment can lead to the activation of intracellular cascades that regulate gene expression and ultimately control neuronal survival and structural plasticity. Chronic administration of omega-3 fatty acids can cause an increase in nerve growth factors, particularly brain-derived neurotrophic factor (BDNF), and these nerve growth factors can play a role in the plasticity and survival of the developed, adult nervous system (23, 24).

Enhancing the cyclic AMP (cAMP) signal-transduction cascade increases the activity and expression of cAMP response element-binding protein (CREB), which in turn increases BDNF (25). Serum BDNF has been found to be negatively correlated with the severity of depressive symptoms (26-30).

Bourre et al. outlined the essentiality of omega-3 fatty acids for brain structure and function and proved that omega-3 fatty acids are essential components of CNS membrane phospholipid-acyl chains and, as such, are critical to the dynamic structure of neuronal membranes (31). Alterations in membrane lipids can alter function by changing fluidity. Proteins are embedded in the lipid bilayer and the conformation or quaternary structure of these proteins appears to be sensitive to the lipid microenvironment. The proteins in the bilayer have critical cellular functions, acting as receptors, enzymes, and transporters (32-36). An optimal fluidity is required for neurotransmitter binding and signaling within the cell (37). Further insight into the molecular basis of action of omega-3 fatty acids was provided by Haag, who suggested that saturated fatty acids have straight carbon chains. Cis-desaturation of a fatty acid has spectacular consequences for its 3-dimensional structure: progressive insertion of cis-double bonds causes the carbon chain to become more curved. The hydrophobic ends of these kinked chains are probably curled around one another in the cell membrane. The more kinked the fatty acid is, the more space it will take up when it is built into cell membrane phospholipids, thereby increasing the fluidity and hence the functionality of the cell membrane (38).

Omega-3 fatty acids can modulate many of the signal transduction mechanisms operating in neuronal membranes and thus in the synaptic cleft. Various neurotransmitters such as serotonin, catecholamines and acetylcholine interact with members of a heptahelical transmembrane receptor family (39-41). The experiments of Murphy et al. (42) on neural cell lines showed that omega-3 fatty acids can increase adenyl cyclase activity. These findings were further confirmed by experiments of Nicholas et al. on pig adipocyte plasma membranes (43). Adenyl cyclase is the enzyme that drives the cAMP messenger system. This pathway is used by 5-HT_1 (serotonin) receptors, alpha-2 adrenergic and beta-adrenergic receptors, and both D_1 and D_2 (dopamine) receptors. As is known that depression is invariably associated with decreased serotonergic neurotransmission, it is evident that the increase in adenyl cyclase activity and hence facilitation of serotonergic transmission by omega-3 fatty acids is contributive to the management of depression. Since fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and raises the synaptic concentration of 5-HT; and signal transduction for 5-HT pathway is operative through cAMP pathway, it seems that fluoxetine exerts augmented effect while acting in combination with omega-3 fatty acids by utilizing this facilitated 5-HT pathway.

The finding that omega-3 fatty acids have antidepressant activity and their combination with fluoxetine has augmented antidepressant activity is significant in view of the fact that fluoxetine and other SSRIs are associated with various serotonergic side effects such as anxiety, insomnia, sexual dysfunction, serotonin syndrome etc. (44), while omega-3 fatty acids are free from any significant adverse effects. Thus, when used in combination with fluoxetine, omega-3 fatty acids may decrease the dose and duration of the SSRI administered. Also when used as nutritional supplements for long term, they may prove beneficial for prevention of depression in susceptible population.

CONCLUSION

Omega-3 fatty acids have antidepressant activity per se, and the combination of fluoxetine and omega-3 fatty acids has more antidepressant efficacy than fluoxetine alone in forced swimming test in Wistar rats.

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