Fluoxetine Potentiation of Omega-3 Fatty Acid Antidepressant Effect: Evaluating Pharmacokinetic and Brain Fatty Acid-Related Aspects in Rodents

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ABSTRACT: We previously reported that combined fluoxetine administration at antidepressant doses renders additive antidepressant effects, whereas non-antidepressant doses potentiate the omega-3 fatty acid antidepressant effect. In the present study, we aimed to evaluate putative pharmacokinetic and brain omega-3 fatty acid-related aspects for fluoxetine potentiation of omega-3 fatty acid antidepressant effect in rats. Coadministration of omega-3 fatty acids with a non-antidepressant dose of fluoxetine (1 mg/kg day) failed to affect both brain fluoxetine concentration and norfluoxetine plasma concentration profile. Fluoxetine plasma concentrations remained below the sensitivity limit of the detection method. Either antidepressant (10 mg/kg day) or non-antidepressant (1 mg/kg day) doses of fluoxetine in combination with omega-3 fatty acids increased hippocampal docosapentaenoic acid (DPA, 22:5 omega-3) levels. Although individual treatments had no effects on DPA concentration, DPA increase was higher when omega-3 were combined with the non-antidepressant dose of fluoxetine. Chronic DPA administration exerted antidepressant-like effects in the forced swimming test while increasing hippocampal docosahexaenoic (22:6 omega-3) and DPA levels. Our results suggest no pharmacokinetic interaction and reveal specific hippocampal DPA changes after fluoxetine and omega-3 combined treatments in our experimental conditions. The DPA role in the synergistic effect of fluoxetine and omega-3 combined treatments will be for sure the focus of future studies. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3316–3325, 2014

Keywords: omega-3 fatty acids; antidepressants; fluoxetine; forced swimming test; depression; drug effects; drug-like properties; pharmacodynamic; preclinical pharmacokinetic; physiological model

INTRODUCTION

Polyunsaturated fatty acids omega-6 and omega-3, as linoleic acid (LA, 18:2) and alpha-linolenic acid (ALA; l8:3), respectively, are essential nutrients for optimal health that must be obtained from the diet as they cannot be synthesized *de novo*.[1] These fatty acids are precursors of omega-6 and omega-3 long-chain polyunsaturated fatty acid families. Among them, arachidonic acid (AA; 20:4 omega-6) and docosahexaenoic acid (DHA; 22:6 omega-3) are highly abundant in the brain[2,3]; whereas others, such as the omega-3 eicosapentaenoic acid (EPA; 20:5), are present at very low levels.[4] In recent years, there has been a growing interest in certain omega-3 fatty acids, such as DHA and EPA in mood disorders. Epidemiological data show a negative correlation between omega-3 fatty acid enriched diet consumption and rates of depression[5–8] as well as omega-3 fatty acid serum levels and the severity of this disease.[9–12] Preclinical evidence has demonstrated the antidepressant effect of omega-3 fatty acids (DHA plus EPA) administered alone[13–17] or in combination with antidepressant drugs such as fluoxetine or mirtazapine.[17] Also, clinical studies have shown the efficacy of omega-3 fatty acid monotherapy[18–21] or combined treatments with antidepressants for major depressive disorder, childhood depression, resistant depression, and bipolar depression.[22,23]

Recent studies in our laboratory have shown that the combined administration of antidepressant doses of fluoxetine and omega-3 fatty acids in rats renders additive antidepressant effects. Moreover, non-antidepressant doses of fluoxetine potentiate the antidepressant effect of omega-3 fatty acids.[17] However, the mechanisms underlying omega-3 fatty acids and fluoxetine synergistic effects in combined treatments are not yet well understood. The aim of the present study was to examine putative pharmacokinetic and brain omega-3 fatty acid-related aspects for fluoxetine potentiation of omega- 3 fatty acid antidepressant effect. To this aim, we evaluated fluoxetine/norfluoxetine plasma and brain levels as well as omega-6 and omega-3 fatty acid levels in the hippocampus and cerebral cortex under omega-3 fatty acids and fluoxetine single or combined chronic treatments.

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EXPERIMENTAL PROCEDURES

Animals and Drugs

Adult male Wistar rats weighing 200–390 g at the beginning of the experiment were used. Three or four animals were housed in polyethylene cages (55 × 38 × 30 cm^3) in a temperature-controlled room (20 ± 1°C) on a 12:12-hour light-dark cycle (8 AM lights on) with free access to food and water except during testing. Animals were used only once in each test. All studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA. The experimental protocols were approved by the Ethics Committee for the Care and Use of Laboratory Animals of the School of Pharmacy and Biochemistry at the University of Buenos Aires. Special care was taken to minimize the number of animals used and their suffering.

All chemical substances were of analytical grade. Fluoxetine–HCl (Gador Laboratory, Buenos Aires, Argentina), norfluoxetine (Sigma–Aldrich, Inc., St. Louis, Missouri), and docosapentaenoic acid (DPA; 22: 5 omega-3; Nu-Chek Prep, Inc., Elysian, Minnesota) were used.

Experimental Design

In the first set of experiments, rats were randomly assigned into six groups: control + saline (c-sal), control + fluoxetine 10 mg/kg (c-flx 10), control + fluoxetine 1 mg/kg (c-flx 1), omega-3 + saline (omega-3-sal), omega-3 + fluoxetine 10 mg/kg (omega-3-flx 10), and omega-3 + fluoxetine 1 mg/kg (omega-3-flx 1). Control animals (c-sal and c-flx) were fed with the standard diet (casein 20%). Omega-3 sal and omega-3 flx rats were fed with standard diet supplemented with fish oil (Tables 1 and 2).

Prior to each experiment, fluoxetine–HCl was dissolved in distilled water and administered by intraperitoneal (i.p.) injection in a volume equivalent to 1 cc/kg. Control groups received daily i.p. injections of saline solution (0.9% NaCl). The antidepressant dose of fluoxetine (10 mg/kg day) was chosen according to previous studies that demonstrated a robust effect in the forced swimming test (FST) employing similar conditions.[17,24,25] The fluoxetine dose lacking antidepressant effect (1 mg/kg day) was also chosen according to previous studies.[17,26] Rats were injected with fluoxetine or saline solution once a day for 16 days and test sessions took place 24 h later (day 17) in accordance with previous studies.[17,27]

To evaluate the possible antidepressant effect of DPA (22:5 omega-3), at the beginning of the study, another set of animals were randomly divided into two groups: rats that orally received DPA (150 mg/kg day) or olive oil (control) for 16 days. The dose of DPA used in the present study was based on a previous report.[28] The weight of the animals was recorded every day for 17 days.

Table 1. Diet Composition

	Control	Omega-3 Fatty Acid Supplemented Diet
	g/kg Diet	
Calcium caseinate	200	200
Corn oil	50	50
Choline chlorhydrate	1.5	1.5
Vitamin mixture[a]	10	10
Mineral mixture[b]	35	35
Maltodextrin	696.9	696.9
Salmon oil	–	11.93

[a]Composition of vitamin supplement triturated in sucrose (g/kg of diet): D-calcium panthotenate, 1.60; nicotinic acid, 3.00; D-biotin, 0.02; menadione, 0.029; thiamine HCl, 0.60; riboflavin, 0.60; folic acid, 0,20; DL-alpha-tocopherol acetate (500 μ/g), 15.00; retinyl palmitate, (400 UI/g), 0.228; pyridoxine HCl, 0.70; cyanocobalamin 0,1% (triturated in mannitol 1:1000), 2.50; cholecalciferol, (250,000 U/g), 0.40; sucrose, 975.123.

[b]Composition (g/kg of diet) as follows: K_2HPO_4, 322.5; $CaCO_3$, 357; NaCl, 74; MgO, 0.8; $MgSO_4.7H_2O$, 146.9; $ZnSO_4.5H_2O$, 0.63; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.008; KI, 0.0078; $Na_2SeO3.5H_2O$, 0.1025; iron and ammonium citrate, 6.06; ZnCl, 1.79; sucrose 91.

Table 2. Fatty Acid Composition of Control and Omega-3-Supplemented Diets[a]

	Experimental Diet	
Fatty Acids	Control	Omega-3 Diet
14:0 Myristic	0.11	7.25
16:1 Palmitoleic	0.16	9.63
16:0 Palmitic	6.75	18.44
18:0 Stearic	3.04	3.51
18:1 Oleic	29.2	14.43
18:2 *n-6* Linoleic	58.8	3.06
18:3 *n-3* Alpha-linolenic	0.11	0.69
20:4 *n-6* Arachidonic	0.24	1.55
20:5 *n-3* Eicosapentaenoic	NC	17.39
22:5 *n-3* Docosapentaenoic	NC	2.68
22:6 *n-3* Docosahexaenoic	NC	12.13

[a]Values are expressed as g/100 g total fatty acids, determined by gas chromatography according to previous studies.[17]

Diet Composition

Omega-3 fatty acids (720 mg/kg day) were administered as a diet supplement in food enriched with salmon oil for 16 days according to previous studies.[17] Each 1000 mg of highly concentrated salmon oil contained approximately 30% omega-3 fatty acids (17% EPA and 13% DHA), rendering 40 mg of salmon oil per gram of the enriched food. Omega-3 fatty acids were added every morning. The amount of diet eaten per day in the different groups was quantified and the average value was employed to calculate the omega-3 dose administered during the 16-day treatment. Diets were equivalent in overall fat, protein, carbohydrate, and caloric content (Table 1).

Total lipid content of the diet was 50 g/100g of wet weight and the three main unsaturated fatty acids present in the control diet were oleic acid (29.2%), LA (58.8%), and ALA (0.11%); whereas EPA, DPA, and DHA were not detected (Table 2).

Forced Swimming Test

Experimental Procedure

In this study, we used the FST, a well-accepted model to test the antidepressant-like action of agents and to identify in rats treatments with antidepressant efficacy in humans.[29] Stress is a well-known risk factor in the development of depression. The FST employs forced swimming stimuli as stressor to generate a behavior characterized by increased immobility time. In our protocol, two sessions were conducted: an initial 15-min pretest on day 1 followed by a 5-min test on day 17. Chronic omega-3 fatty acids and/or fluoxetine treatment began on day 1 after the pretest session.[17] Swimming sessions were conducted by placing rats in individual Plexiglas cylinders (46 cm tall × 20 cm