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Research report

Antidepressant-like effects of omega-3 fatty acids in postpartum model of depression in rats

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HIGHLIGHTS

- The first evaluation of antidepressant effect of omega-3 on postpartum depression model of rat.
- The first measurement of pro-inflammatory cytokines in postpartum-induced rats.
- The first evaluation of impact of omega-3 on pro-inflammatory cytokines in postpartum-induced rats.
- The first evaluation of effect of omega-3 on corticosterone levels in postpartum-induced rats.

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ABSTRACT

Postpartum depression (PPD) is a psychiatric disorder that occurs in 10–15% of childbearing women. It is hypothesized that omega-3 fatty acids, which are components of fish oil, may attenuate depression symptoms. In order to examine this hypothesis, the animal model of postpartum depression was established in the present study. Ovariectomized female rats underwent hormone-simulated pregnancy (HSP) regimen and received progesterone and estradiol benzoate or vehicle for 23 days, mimicking the actual rat's pregnancy. The days after hormone termination were considered as the postpartum period. Forced feeding of menhaden fish oil, as a source of omega-3, with three doses of 1, 3, and 9 g/kg/d, fluoxetine 15 mg/kg/d, and distilled water 2 ml/d per rat started in five postpartum-induced and one vehicle group on postpartum day 1 and continued for 15 consecutive days. On postpartum day 15, all groups were tested in the forced swimming test (FST) and open field test (OFT), followed by a biochemical assay. Results showed that the postpartum-induced rats not treated with menhaden fish oil, exhibited an increase in immobility time seen in FST, hippocampal concentration of corticosterone and plasmatic level of corticosterone, and pro-inflammatory cytokines. These depression-related effects were attenuated by supplementation of menhaden fish oil with doses of 3 and 9 g/kg. Moreover, results of rats supplemented with menhaden fish oil were comparable to rats treated with the clinically effective antidepressant, fluoxetine. Taken together, these results suggest that menhaden fish oil, rich in omega-3, exerts beneficial effect on postpartum depression and decreases the biomarkers related to depression such as corticosterone and pro-inflammatory cytokines.

1. Introduction

Postpartum depression (PPD) is a psychiatric disorder, defined as a subtype of major depressive disorder (MDD). It has been reported that 10–15% women suffer from PPD following childbirth [1]. Although the underlying etiology of postpartum depression remains unknown, the abrupt changes in reproductive hormones that women undergo in post-delivery period may cause postpartum depression [2]. In addition to the role of estrogen and progesterone, some other biological factors such as hypothalamic–pituitary–adrenal (HPA) axis hormones, altered immune system and cytokines, and altered fatty acid have been proposed to play a role in causing postpartum depression [3].

Not diagnosing and treating postpartum depression has significant adverse effects on depressed individuals and their families [4]. Due to side effects of medical treatment on breastfed infants and the

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negative effects of untreated depression, mothers face a dilemma about how to deal with depression symptoms. Therefore, another alternative treatment should be considered to lessen depressive symptoms with lower side effects for both mother and baby.

The relationship between omega-3 and depression has been reported in previous studies. There are many reasons indicating an inverse relationship between omega-3 fatty acids and depression. This link has been observed in both observational and experimental research [5,6]. Fish and fish oil, which are rich in omega-3 fatty acids, are the best dietary sources of omega-3 [7].

Although it is believed that omega-3 plays a vital role in the body, and in particular, in the nervous system and mood disorder, the underlying mechanism is poorly understood. While numerous studies have been carried out to evaluate the effects of omega-3 on depression and other mood disorders, the researches carried out to determine the effects of omega-3 on postpartum depression are few and the results are inconsistent.

Due to the contradictions among the results of previous researches in this regard, the present study was performed to clarify these discrepancies by investigating the effects of menhaden fish oil (rich in omega-3) on postpartum-induced rats. In order to study the antidepressant-like effects of omega-3 on postpartum-induced rats, a hormone manipulation was used to establish the animal model of postpartum depression, and then these PPD-induced rats were supplemented with menhaden fish oil. The antidepressant-like effect of omega-3 was evaluated using a standard behavioral test (FST) and also the effect of omega-3 on HPA axis was assayed by measuring corticosterone levels in the plasma and hippocampus of PPD-induced rats. Moreover, to determine the relationship between omega-3 and immune system responses, the plasma levels of pro-inflammatory cytokines were also measured.

2. Materials and method

2.1. Subjects

Forty-two female Sprague-Dawley rats (60–70 days old, weighing 180–200 g at the beginning of the experiment), obtained from Saintik Enterprise Company, were used in this study. Animals were kept in Animal House, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. They were housed 3 per cage under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 am, in a temperature of $22 \pm 1\,^{\circ}C$ and with free access to water and standard rat food pellets. Animals were allowed 14 days to acclimatize to the laboratory conditions prior to the experiment. Experiments were conducted between 8:00 a.m. and 3:00 p.m. All procedures involving animals were conducted in accordance with ethical guidelines and with approval from the Animal Care and Use Committee (ACUC), Faculty of Medicines and Health Sciences, Universiti Putra Malaysia. In order to minimize animal suffering, the minimum number of animals and duration of observations were employed to gain reliable data.

2.2. Surgery

At the beginning of the experiment, rats were ovariectomized (OVX). Using an aseptic technique, the ovariectomy was carried out bilaterally under anesthesia with xylazine 10 mg/kg and ketamine 80 mg/kg i.m. Ovariectomized rats were allowed to recover for one week following surgery, while their surgery scars were closely observed daily [8].

2.3. Hormone simulating pregnancy (HSP)

After one-week's rest following the ovariectomy procedure, hormone regimens were started. Ovariectomized rats were administered hormones (estradiol and progesterone), dissolved in 0.1 ml sesame oil, for 23 days to establish hormone simulated pregnancy (HSP) as shown in Table 1. The duration and doses of the HSP regimen mimicked of the rat's normal gestation and were chosen based on previous studies reporting that this amount of hormones can efficiently produce maternal behavior in nulliparous female rats [8–12]. This study included five HSP groups, one vehicle group, and one normal control group. The vehicle group was subcutaneously injected with sesame oil (vehicle) after ovariectomy, with the same volume and duration of hormones. Rats in the normal control group were virgin female rats, which had no experience of ovariectomy and injection. In order to determine whether the HSP-treatment produced maternal behavior (and whether the vehicle treatment did not), all rats were placed individually in cages (one rat/cage) after injection on day 23 of HSP regimen where they were provided with paper towels as nesting materials. After 24 h, each cage was observed for the presence or absence of nesting behavior. Maternal behavior was scored positive when rats made paper towels shredded into small pieces and arranged them into a circular nest in one corner of the cage. The absence of nesting behavior was considered as negative maternal behavior [8].

Table 1
Hormone-simulated pregnancy (HSP) regimen.

Groups	Days 1–16	Days 17–23
HSP	Estradiol benzoate (2.5 μg/rat) + Progesterone (4 mg/rat)	Estradiol benzoate (50 μg/rat)
Vehicle	Sesame oil (0.2 ml/rat)	Sesame oil (0.1 ml/rat)

2.4. Supplementation protocols

Based on the fact that the amount of food consumed varies for each rat, oral administration of fish oil through gavage was chosen to ensure sufficient amount of omega-3 received by each rat. After termination of hormones and vehicle injection, rats which were fed on the normal diet, were given different forced feed regimens as described below for 15 consecutive days (Fig. 1). The amount of menhaden fish oil (Sigma F8020) administered was calculated daily after weighing rats, using the formula [13] as below:

for dose of 1 g/kg: (body weight (g) × 0.001)/density (g/ml)
for dose of 3 g/kg: (body weight (g) × 0.003)/density (g/ml)
for dose of 9 g/kg: (body weight (g) × 0.009)/density (g/ml)

According to the rats' weights, which were in the range of 200–230 g, and the menhaden fish oil's density that was 0.93 g/ml, the maximum amount of oil administered was not more than 2.2 ml/gavage. This amount of fish oil was high enough to show effects without causing diarrhea or stomach regurgitation. Menhaden fish oil, which includes approximately 30% omega-3 fatty acids, has been previously used as the source of omega-3 supplementation in animal [14] and human [15] studies.

2.5. Behavioral tests

2.5.1. Forced swim test (FST)

In order to determine whether this animal model of postpartum induces depression-like behavior and whether omega-3 may attenuate this depression, an FST was conducted on postpartum day 15 followed by open field test (OFT). In order to minimize the number of rats used, rats exposed to the open field test were also tested in the forced swimming test (approximately 1 h between tests). Basically, the apparatus of the FST is a rectangular tank (25 cm × 25 cm w × 60 cm h) containing water at temperature of $27\,^{\circ}C$ ($\pm 2\,^{\circ}C$) and 30 cm deep [16]. On postpartum day 14, pre-test of FST was conducted. Animals were placed in the swim tank individually for 15 min to induce a state of helplessness.