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Effect of omega-3 polyunsaturated fatty acid treatment over mechanical allodynia and depressive-like behavior associated with experimental diabetes

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HIGHLIGHTS

- Fish oil prevents the mechanical allodynia in diabetic rats.
- Fish oil exerts an antidepressant-like effect in diabetic rats.
- Fish oil restores the BDNF levels in the hippocampus and cortex of diabetic rats.

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ABSTRACT

Neuropathic pain and depression are very common comorbidities in diabetic patients. As the pathophysiological mechanisms are very complex and multifactorial, current treatments are only symptomatic and often worsen the glucose control. Thus, the search for more effective treatments are extremely urgent. In this way, we aimed to investigate the effect of chronic treatment with fish oil (FO), a source of omega-3 polyunsaturated fatty acid, over the mechanical allodynia and in depressive-like behaviors in streptozotocin-diabetic rats. It was observed that the diabetic (DBT) animals, when compared to normoglycemic (NGL) animals, developed a significant mechanical allodynia since the second week after diabetes induction, peaking at fourth week which is completely prevented by FO treatment (0.5, 1 or 3 g/kg). Moreover, DBT animals showed an increase of immobility frequency and a decrease of swimming and climbing frequencies in modified forced swimming test (MFST) since the second week after diabetes injection, lasting up at the 4th week. FO treatment (only at a dose of 3 g/kg) significantly decreased the immobility frequency and increased the swimming frequency, but did not induce significant changes in the climbing frequency in DBT rats. Moreover, it was observed that DBT animals had significantly lower levels of BDNF in both hippocampus and pre frontal cortex when compared to NGL rats, which is completely prevented by FO treatment. In conclusion, our study demonstrates that FO treatment was able to prevent the mechanical allodynia and the depressive-like behaviors in DBT rats, which seems to be related to its capacity of BDNF level restoration.

1. Introduction

Diabetes is a chronic clinical condition [1] that, according to the International Diabetes Federation, affects importantly about 387 million people worldwide, a number expected to rise to about 590 million people in 2035 [2]. Among the diabetic complications, the diabetic sensorimotor polyneuropathy is the most common clinical form of diabetic neuropathy, affecting around 90% of patients with diabetes [3,4]. Depending on diagnostic criteria, about 10–46% of the patients with diabetic neuropathy develop neuropathic pain along the disease [5,6]. Additionally, it has been described that diabetic patients have 15–20% higher chances to develop depression when compared to the general population [7,8].

Studies suggest also a bidirectional relationship between diabetes and depression, being the major depression the most

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prevalent mood disorder in diabetics [9,10]. On the other hand, patients with installed depressive symptoms present an increased in fasting blood glucose levels [11] and an increased risk of develop diabetes [12]. There is also a bidirectional correlation between chronic pain and depression, i.e. patients with chronic pain have higher incidence of depression when compared with the general population [13]. Similarly, the chances to develop chronic pain is higher in the presence of depressive symptoms [14].

The search for alternative therapies for the treatment of neuropathic pain [15] and depression associated with diabetes is recquired, since the conventional therapies present low adhesion [16], responsiveness [4] and may also alter blood glucose levels [17,18]. In this way, omega-3 polyunsaturated fatty acids (ω-3-PUFA), as linolenic acid (LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been suggested as adjuvant therapy of depression [19–21], pain [22–24] and also for mood disorders [25,26], among others [for review see: Ref. [27]]. Regarding the possible mechanism of action of ω-3-PUFA, especially in regard to its antidepressant-like effect, it has been associated with a hippocampal brain derived neurotrophic factor (BDNF) up-regulation [28,29] and also with other biological functions [30].

Although the antinociceptive and the antidepressant effects of ω-3-PUFA have already been observed previously, to our knowledge the effectiveness of this teatment in depressive-like behaviors or in the neuropathic pain associated with diabetes has not been tested yet, which was the main goal of this study. More specifically, our aim was to investigate the effect of fish oil, a natural source of EPA and DHA, over the mechanical allodynia and depressive-like behaviors in a rat model of type 1 diabetes induced by streptozotocin (STZ), exploring whether alterations in BDNF levels may be implicated in its mechanism of action.

2. Material and methods

2.1. Animals

Experiments were conducted in male Wistar rats (weighing 160–170 g), provided by Federal University of Paraná colony. The animals were maintained in the vivarium of Department of Pharmacology, under controlled conditions of temperature and light/dark cycle of 12 h, with free access to food and water. The animals were divided into 3 three experimental groups of 6–9 rats each (Normoglycemic vehicle, Diabetic treated with vehicle and Diabetic treated with fish oil). The normoglycemic animals were maintained at about 5–6 animals per cage, while the diabetic animals were kept in smaller number per cage (3–4 animals, with shavings changed daily) for better accommodation and comfort, as they clearly develop signs such as polydipsia and polyuria. All experiments were conducted in accordance with the rules and laws contained by the Ethics Committee for Research on Animals UFPR (CEUA/BIO-UFPR; #790). All efforts were made to minimize the number of rats and their suffering.

2.2. Drugs

The following drugs were used: fish oil (FO, kindly donate by Laboratório Herbarium Botânico S/A, Colombo, Parana, Brazil) composed of 26% EPA and 20% DHA of total fatty acids [31], and streptozotocin (STZ, Santa Cruz Biotechnology Inc., USA). The fish oil was administered orally (by gavage) at doses of 0.5, 1 or 3 g/kg during 8 weeks. Streptozotocin was dissolved in citrate buffer (10 mM, pH 4.5) and administered intraperitoneally (i.p.) at a dose of 60 mg/kg. The doses, routes and treatment schedules were established based on previous studies from our group [32,33] or other studies [34,35].

2.3. Diabetes induction

Diabetes was induced experimentally by a single intraperitoneal injection of 60 mg/kg of streptozotocin (STZ) (Sigma–Aldrich) dissolved in citrate buffer (10 mM, pH 4.5), i.p, in rats previously fasted for 12 h. Normoglycemic group, a control group run in parallel, received only citrate buffer (10 mM, pH 4.5, equivalent volume). Diabetes was confirmed three days after the injection of STZ using samples of about 5 μL of blood from the tail vein added to test strips impregnated with glucose oxidase (Accu-Check ActiveTM, Roche). The animal was considered diabetic and kept in the study when glucose was equal or greater than 250 mg/dL. Body weight was determined weekly.

2.4. Mechanical allodynia measurement (electronic Von Frey test)

The mechanical allodynia was assessed using an electronic Von Frey (electronic anesthesiometer, IITC Life Science, CA, USA), as earlier described by [36]. Briefly, the device presents a disposable polypropylene tip (0.5 mm in diameter), fixed in a force transducer connected to a digital display, where is possible to verify the force (g) applied in the medial surface of animal hind paws. Plexiglass boxes placed in a wire mesh floor (thickness of 5 mm^2) were used to acclimate rats during 15 min, or time enough to cease exploratory behaviors. Through the mesh spaces was applied linearly growing force until the occurrence of a withdrawal response and "shake/licking" of the stimulated paw. The mechanical threshold of a single paw were calculated by the average value of three similar withdrawal responses while the average mechanical threshold of an animal were calculated as the average mechanical threshold of both hind paws. The presence of mechanical allodynia was obtained by the difference among average mechanical threshold observed prior to administration of STZ (baseline or zero time) in comparison of different time points after induction of diabetes or after fish oil administration.

2.5. Modified forced swimming test

The modified forced swimming test was conducted in the second and again at fourth week after STZ injection, following the procedures originally described by [37] and modified by [38]. Briefly, the animals were placed individually in plastic swimming cylinders (20 cm diameter × 50 cm containing 30 cm of water at a temperature of 24 ± 1 °C) for 15 min (pre-test). Twenty-four hours after the pre-test, the animals were submitted to a session of 5-min forced swim (test session) which was recorded for subsequent evaluation of the frequency of immobility (except small movements needed to float), swimming and climbing every 5 s. This behavioral despair test characterize the depressive-like behavior as a reduction in the frequency responses of active (swimming and climbing) and an increase in the frequency response of passive (immobile). Between an animal and another, clean water was restored after the cylinder be cleaned properly. After each session (pre-test and test), the animals were removed and subjected to drying with clean and dry cloths in a separate box before returning to their home cages.

2.6. Open field test

The open field test was performed as previously described [39] Briefly, the animals were placed in the center of a rectangular apparatus (40 × 50 × 63 cm) with a floor divided into 6 rectangular units. The behavior was video recorded during 5 min and the number of squares crossed with all four paws was considered a measure of the locomotor activity. Then the animals returned to their cages and the apparatus was cleaned with alcohol 20% prior to testing in other animal. The data obtained after different treatments were