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Original Research

Beneficial effects of deep sea fish oil on diabetic mice neurological injury

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Abstract: Deep sea fish oil mainly includes polyunsaturated fatty acid, which is usually used as health products and applied for prevention of cardiovascular and cerebrovascular diseases. However, there are only a few studies investigating the diabetes induced nerve injury till now. We established the diabetic model by using the 8-week old inbred male mice, and assumed that fish oil had a certain therapeutic effect on related neurosensory impairment and oxidative stress. Mice were divided into group A (diabetic mice induced by streptozotocin (STZ) and treated with fish oil), group B (diabetic mice) and group C (normal mice, without STZ treatment). The memory and exploration ability were evaluated and oxidation status of brain tissue was detected. Results indicated that memory and exploration ability of fish oil group A was significantly improved compared to diabetic group B ($P<0.05$), and equal to group C. The malondialdehyde (MDA) level of fish oil group A was decreased significantly and antioxidant level was increased significantly compared to diabetic group B ($P<0.05$), and equal to group C. In conclusion, deep sea fish oil could be used as auxiliary health care products, which plays important role in preventing and treating implications of nerve lesion impairment induced by diabetes mellitus.

Key words: Deep sea fish oil; Diabetes mellitus; Nervous lesion.

Introduction

Diabetes mellitus is a kind of systemic metabolic disorder. Clinical and experimental studies showed that there was diabetes related neurosensory impairment in the hippocampus and neurodegenerative changes in the diabetic mice. However, the diabetes related cognitive impairment was always affected by many factors. At present, the pathogenesis remains unclear but specific factors could be involved, such as the level of hyperglycaemia, insulin deficiency, increased oxidative stress, hyperactivity of the hypothalamic pituitary axis, activation of inflammatory response pathways (1-4). The deep sea fish oil is abstracted from the fish in the deep sea. Deep sea fish oil is a dietary supplement that contains a nutrient called omega-3 fatty acids essential to bodily functions. Clinical trials have been shown that fish oil treatmentimproves cognitive function of patients possibly on Alzheimer disease and other neurodegenerative diseases (5-10).

Polyunsaturated fatty acid plays an important role in lipid derived cell signaling, gene expression and inflammatory processes. The deep sea fish oil was unable to be synthesized in human body and can only be supplemented by diet. Deep sea fish oil contains docosahexenoic acid (DHA), eicosapentaenoic acid (EPA), α-linolenic acid (ALA) and other essential nutrients, which can promote nerve growth and development for mammals. It also can promote the formation of spinous process, activate the cell anti-apoptotic pathway and significantly improve the study and memory ability (11). Some studies pointed out that deep sea fish oil could protect the glutamate nerve transfer process from injury, so that prevent the occurrence of stress related depression and anxiety neurosis. Glucocorticoid receptor might participate in memory and learning process in hippocampus (12-14). Oxidative stress results from either a decrease of natural cell antioxidant capacity or an increased amount of reactive oxygen species (2,3). The malonaldehyde and the total antioxidant activity could reflect the oxidative stress status. Therefore, in this study, we used the diabetic mice to explore the effects of deep sea fresh oil on the associated neurosensory impairment and the oxidation status, which provides a certain reference value for clinical diagnosis and treatment.

Materials and Methods

Establishment of animal model and trial grouping

Total of 50 inbred male mice (8-week old) were purchased from Beijing Meisen biological medicine science and Technology Co. Ltd.. The body weights range from 30 g to 35 g, The mice were divided into 3 groups: group A (20 mice for fish oil group), group B (20 mice for simple diabetes group) and group C (10 mice for control group). All the animals were raised alone under standard animal feeding condition. All the operations for the mice in this study were agreed by the ethics committee of Shanxi province people's hospital, Xi'an, China. The streptozotocin (STZ, 45 mg/kg) was dissolved in 5 mM sodium citrate buffer (pH 4.5), and was intraperitoneally injected into the mice in group A

and group B, respectively. Only injects equal dose of PBS buffer to the mice in the control group C. In order to prevent the occurrence of acute hypoglycemia, a 5% glucose solution was intraperitoneally injected into the mice 12 h after STZ treatment. The blood sugar level was tested by using a blood-glucose meter for testing in the 3rd and 10th day after giving STZ, a 300 mg/dl high blood sugar level was constant for the next 20 days.

Deep sea fish oil

The deep sea fish oil used in this study was purchased from Doils® joints (Nutraceuticoils, Belgium), and was treated by using the method described in a previous study (19) . According to the product description of deep sea fish oil, it includes 10.32 kcal energy, 0.19 g carbohydrates, 0.24 g protein, 0.95 g fattiness, 30 IU D-α-tocopherol, 1030.50 mg salmon oil and omega-3 (which consists of 180 mg EPA and 120 mg DHA; Doils® joints, Nutraceuticoils, Belgium). The total fatty acid of a single capsule was about 500 mg, and 18% was EPA, 12% was DHA. The daily dosage used in this study consists of 0.5 g/kg, and orally done at 10 a.m.

Detection of behavior activity

After taking deep sea fish oil for 30 days, the open-field test (OFT) and passive avoidance were used to evaluate the anti-anxiety activity and its memory ability. At present, the open field test (OFT) is the method which has been widely used to evaluate the exploratory activity and emotional response of rodent animals. The specific step was to put the mice in a bright environment that was enclosed by a border (100×100×40 cm). The bottom contains 25 square frames, and placing a 100 w lamp bulb 60 cm at the top of the space center for lightening. The following parameters were recorded and quantified, including the number of walk, hind limb standing, the movement of the center position and the stool excretion. A wooden box with light and dark zone was used for passive avoidance test. The bottom of the box was a grid floor connected with electrical stimulation device. The mice were allowed to explore these two zones for 5 min at the first day. Then, through 3 rounds of 5 min test, recording the time when mice stayed in each side. For the 4th test, a 2.5 mA electric shock was given when mice entered into the dark zone, and then sent them back to their cages. After 24 hours, the mices were placed into the zone again and the delay time to enter into dark zone was also recorded.

Molecular and biological activity examination for brain tissues

The whole brain tissue was cut into slices. Weighting brain tissues, dipping them in low temperature phosphate buffer solution (PBS, pH 7.4) and the obtaining 10% (weight/volume) tissue bomogenate. Subsequently, abstracting and storing the liquid supernatant at the temperature of -80°C for reserve. The degree of lipid peroxidation in brain tissue was evaluated by thiobarbituric acid (TBA) reagent. The malonaldehyde (MDA) could be obtained from the decomposition of unsaturated fatty acids. It could be regarded as a index number, which reflects the degree of lipid peroxidation. Put it under the 532 nm spectrophotometer and red the number after reacting with TBA. The total antioxidant activity in brain tissue homogenate was examined by using the method that reported previously. The total antioxidant activity was detected under the 532 nm spectrophotometer, and the value was red after reacting.

Data analysis

All the data were analyzed by using SPSS 16.0. The data were expressed as average value ± standard deviation. We applied factor analysis of variance for groups, and used post hoc detection to compare within group. Because passive avoidance test and MDA level did not belong to the normal distribution, then we adopted Kruskall Wallis for detecting. $P<0.05$ represents the statistical differences.

Results

Open field exploration test

In the single factor variance analysis of an open field, there were significant differences among the number of through neighboring squares, through the central square and hind limb standing, respectively ($P<0.05$). There was no significant difference in sorting and defecation scores. Post hoc test mentioned that the average number of entering neighboring square of the mice in diabetic group B (20 mice) was significantly lower compared to control group C (20 mice) ($P<0.05$), and the fish oil group A (20 mice) was significantly compared to the diabetes group B ($P<0.05$). There was no significant difference between normal group C and fish oil group A ($P=1.82$). The average score of entering central square for the mice in diabetes mellitus group B was significantly lower compared to normal group C ($P<0.05$). Meanwhile, the average score of fish oil group A was significantly higher compared to the diabetes group B ($P<0.05$). There was no statistical significance between fish oil group A and normal group C. The number of standing on the hind legs for diabetes mellitus group B was significantly lower than that of normal control group C and fish oil group A ($P<0.01$), and there was no statistical significance between fish oil group A and normal group C (Figure 1).

Passive avoidance test

The obvious time delay of each group existed when the mice entered the dark area the next day was detected by Kruskall Wallis test ($P<0.05$). The post hoc test showed that the delayed times in diabetes group B were lower significantly compared to the normal group C (Figure 2, $P<0.05$), and the delayed times in fish oil group

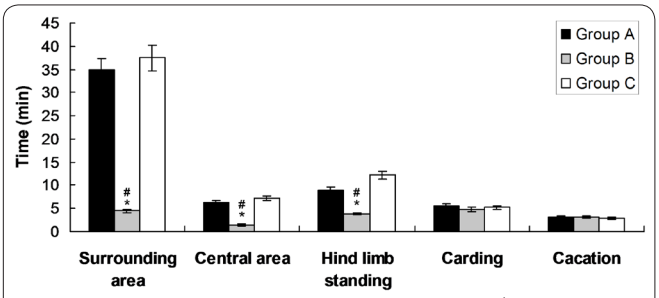

Figure 1. Open field test results in three groups. * $P<0.05$ represents the times in Group B (20 mice) compared to the Group C (20 mice). # $P<0.05$ represents the times in Group B compared to the Group A (10 mice).

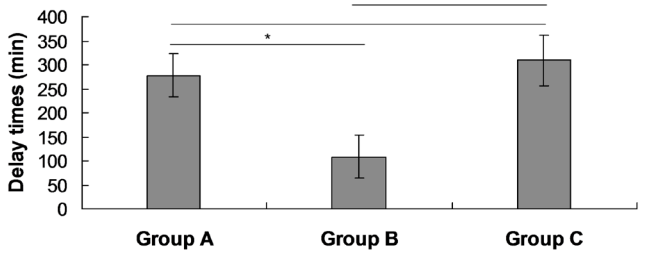

Figure 2. The results of passive avoidance test, *P<0.05 means the different values between the two groups.

A were significantly higher compared to the diabetes group (P<0.05). Furthermore, there were no significant differences for the delayed times between fish oil group A and normal group C (Figure 2, P>0.05).

Biochemical examination of brain tissue homogenate

Kruskall Wallis test showed obvious differences existed among groups (P<0.05). Post hoc tests results showed that there were no significant differences for MDA between diabetes group B and normal group C. The brain tissue MDA level of fish oil group A was significantly lower compared to diabetes group B and normal group C (Table 1, P<0.05). Analysis of total antioxidant levels showed that there was a significant difference in univariate variance analysis groups (F(2, 15)=21.97, P<0.001). Total antioxidant activity of diabetes group B was lower significantly compared to the normal group C (Table 1, P<0.05). The antioxidant activity of fish oil group A was significantly higher compared to the diabetes group B (Table 1, P<0.05). There was no significant difference between fish oil group A and normal group C (Table 1, P>0.05).

Discussion

In this study, we used an EPA and DHA supplementation from deep sea fish oil to explored its effects on diabetes associated neurological impairment and oxidative stress. Neurological improvements by fish oil were shown at two levels in the mice with the sensor motor ability and the memory. The results obtained from fish oil treatments showed that the impaired sensory motor ability induced by diabetes was significantly improved by fish oil treatment to a level similar to control mice. These results are consistent with the previous reports (15). The altered open field exploration ability of the mice was also corrected by treating with the fish oil. This result could be related to the effects of fish oil that antagonizes the anxiety activity and loss of memory evidenced in diabetic mice.

Some studies showed that risk factors for mental illness include the correlation between depression, anxiety and different forms of diabetes mellitus (16-19).

Table 1. Biochemical examination of brain tissue homogenate.

	Group A	Group B	Group C
Malonaldehyde (μmol/L)	0.88±0.32*	2.89±0.15	2.78±0.23
Total antioxidant activity (mmol/L)	6.46±0.14	4.08±0.27*	6.12±0.25

*P<0.05 means compared with other two group, the differences have statistical significance.

The previous study reported that the deep sea fish oil are composed of higher content of ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), however, the amounts of EPA and DHA in common fish oil are very limited (20). Our results are equal to the beneficial effects of a fish oil pharmaceutical formulation (MaxEPA) similar in the ω-3 EPA and DHA content used in diabetic rats (21). In our study, we evidenced a mechanism of action by investigating the antioxidant activity of the deep sea fish oil supplementation in diabetes mice. Our study preliminarily confirmed that the deep sea fish oil could be regarded as a dietary supplement for diabetes therapy, by decreasing the risk of oxidative stress. But the cause and curative effect of diabetes mellitus and multiple sensory impairment are still very complicated, which needed to be confirmed by further basic and clinical trials. There was still controversy about the food therapy for patients with diabetes, but it was reasonable for the patients with neurosensory impairment to choose deep sea fish oil as the supplement and substitute for treatment. Although it was not clear about the specific mechanism of diabetes related nerve damage, the diabetic rats induced by streptozotocin had been shown that the inhibition of serotonin function in different brain regions. It could be reversed by insulin replacement therapy (22-24). The animal experiments showed that long lasting hyperglycaemia could decrease serotonin levels in rat brain and increase the expression of 5-serotonin receptor. This change may also play an important role in diabetes related behavioral abnormalities.

Studies showed that reactive oxygen species (ROS) produced by diabetes mellitus could lead to micro-vascular disease and increase oxidative stress. Therefore, the ROS plays an important role in the occurrence and development of diabetic complication. Oxidative stress causes the damages of neurosensory cells in brain tissues, which led to the decreased morphology and memory ability. It was reported that antioxidants like melatonin and Vitamin E play a protective role under the experimental neural degeneration conditions, which further prevent the diabetes related learning and memory decline (25). In this study, we also found that fish oil could obviously decrease the MDA level in brain tissue homogenate. Actually, the MDA is a product of lipid peroxidation (LPO), and has been adopted as a measure of free radical production. The MDA level was decreased in the fish oil treated group A compared to the diabetes group B. Therefore, the MDA level was also improved, and which could reflect the enhanced antioxidant activity indirectly. Moreover, in the preliminary experiments, we investigated the effects of fish oil on vitamin E level, however, no effects were found on the Vitamin E (data not shown).

In addition, total antioxidant activity (TOA) in this study was increased, which indicated the importance of adding fish oil to the diabetic patients diet. Unsaturated fatty acid in deep sea fish oil could decrease sensibility of nervous tissue on lipid peroxidation, which could produce advantages. It is the source of natural antioxidants, and also plays an important role in the activation of brain-derived neurotrophic factor (BDNF) and NR2B subunit receptor. Because of the low content of antioxidase in nervous tissue, it was important to increase its level for preventing free radicals that produced by dia-

betes mellitus. Cosar and others researches found obvious improvement of hippocampal function after using fish oil for 8 weeks (26), which results were consistent with the result of our study.

In this study, we confirmed that decreased sensory motor ability in diabetic mice, and evidenced that a deep sea fish oil for 1 month is effective. Therefore, it would be helpful to add deep sea fish oil to the supplementary diet for patients with chronic diabetes mellitus for neurological purpose.

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