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EFFECTS OF DOCOSAHEXAENOIC ACID (DHA) IN A MOUSE NEUROINFLAMATION MODEL: BEHAVIORAL AND HISTOLOGICAL ASPECTS

Choupette Ravelle Dobhat-Doukakini[1,2]*, Landry Martial Miguel[1], Childérick Lekana[1,2], Destin Maurélien Mbemba Bahamboula[1,3], Donatien Moukassa[1] and Ange Antoine Abena[1,2]

[1]Clinical and Molecular Biochemistry Unit, Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Republic of Congo.
[2]Licence Department, Denis Sassou- N'Guesso University, Kintélé, Republic of Congo.
[3]Molecular Biology Laboratory, Outpatient treatment Centre, Republic of Congo.

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***Corresponding Author: Choupette Ravelle Dobhat-Doukakini**
Clinical and Molecular Biochemistry Unit, Faculty of Health Sciences, Marien Ngouabi University, Brazzaville, Republic of Congo.

SUMMARY
Neuroinflammation is described as a key mechanism in the onset and/or progression of several neurological disorders and is the subject of several researches. Several protocols have been proposed to model this condition, such as LPS injection, which induces neurodegeneration and anxiety-like behavior. Numerous studies have been carried out to understand the pathophysiological mechanisms of neuroinflammation. The aim of this study was to evaluate the effects of DHA administration on behavioral disorders as well as tissue damage induced by LPS in mice. Thirty male Balb /c strain mice weighing between 11.7 and 30.2g were randomly divided into three groups (n=10). The first group received (distilled water, 1ml/Kg). Neuroinflammation was induced in groups 2 and 3 by administration of 500 µg/Kg, *ip* of LPS for one week. Twenty-four hours after induction of neuroinflammation, the animals received water and DHA (55 mg/kg, per os), respectively. Finally, twenty-four hours after DHA treatment, the animals were sacrificed and the brains collected for histological analysis. The results obtained show that LPS significantly reduces motor activity. However, the administration of DHA at a dose of 55 mg/kg does not significantly increase this activity. Concerning the anxiety index, the results show a non-significant increase in the latter after treatment with DHA. Histologically, our behavioral results are in agreement with histological examination, which revealed that tissues from DHA-treated mice exhibited neurons and macrophage cells containing a pale vesicular nucleus and an obvious nucleolus (in the form of a perinuclear halot), as well as dark, polymorphonuclear cells, degenerating with phagocytic effect, unlike the tissues of the other two groups. In conclusion, our results suggest that administration of 55 mg/kg of DHA has no significant effects on behavior, but histological examination shows progressive repair.

KEYWORDS: LPS, Docosahexaenoic acid, neuroinflammation, histology, behavior.

INTRODUCTION

Neuroinflammation is the common denominator of neurological disorders. It is inflammation of the brain and neurons that make up the nervous system. Several events can lead to neuroinflammation, including infections, brain trauma, toxic metabolites, aging and autoimmune diseases. Neuroinflammation has been described as a key mechanism in the onset and/or progression of several neurological disorders defined as inflammatory (multiple sclerosis, vasculitis, etc.), but also in neurological disorders that are generally not classified as inflammatory, such as Alzheimer's disease, Parkinson's disease, stroke and traumatic brain injury.[1-4] It therefore represents a mechanism of defense and protection of the central nervous system (CNS) in the face of an attack which can also lead to significant damage to neurons when it is not regulated.[5][6] In the brain, inflammatory cytokines are produced by endothelial and glial cells.[7][8] Activation of glial cells and complement-mediated pathways, synthesis of inflammatory mediators as well as oxygen radicals, and leukocyte recruitment are characteristic features of neuroinflammation.[9][10][11]

Furthermore, several models of induction of neuroinflammation in rodents have been proposed, among others, induction by administration of lipopolysaccharides (LPS) and 24-hour restraint.[12][13]

LPS model is the most used to induce neuroinflammation.[14] Increasing evidence suggests that peripheral administration of LPS disrupts brain signaling, leading to deficits in behavior, learning, and memory.[13][15] Furthermore, several authors have reported that certain neurodegenerative disorders were corrected by the administration of various substances such as shikimic acid, hydrogen sulfide and red.[16][17][18]

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA) and structural constituent of membranes, particularly in the central nervous system. Most evidence for the health benefits of (n-3) PUFA is based on studies of supplementation of both eicosapentaenoic acid (EPA) and DHA in different proportions.[19][20][21][22][23] Some data suggest that PUFA intake (n=3) stimulated the development of the immune system in infants.[24] Also, their metabolites may have an anti-inflammatory role in psychiatric, neurodegenerative and neurological disorders.[22]

Recent data support the hypothesis of an inhibition of tumor growth and the metastatic potential of ovarian cancer by a diet rich in DHA.[25] DHA could therefore contribute to creating optimal conditions in cognitive decline or behavioral deficits in certain psychiatric disorders, as well as the repair of tissue damage in the brain in neurodegenerative diseases. The present study aimed to evaluate the effects of docosahexaenoic acid administration on the anxiety-like behavioral profile and brain neuronal damage in a model of LPS-induced neuroinflammation.

MATERIAL AND METHODS

1. Animals

The experiments were carried out on male mice of the Balb /c strain weighing between 11.7 and 30.2g. They came from the animal facility of the Faculty of Health Sciences of Marien NGOUABI University. They were raised in polypropylene cages, under an ambient temperature of 25°C and a light/dark cycle of 12/12, with free access to water and food. All experiments were conducted in compliance with Directive 2010/6106/EU, relating to the protection of laboratory animals. (Hartung, 2010).

2. Chemicals, preparation and administration

DHA (DHA CAS: 6217-54-5) and LPS (*Escherichia coli* serotype O55: B5) were purchased from SOLARBIO INTERNATIONAL BIOTECH®., China. Ten (10) mg of DHA had been dissolved in 1 ml of distilled water. LPS was dissolved in sterile 0.9% isotonic saline without endotoxin before injection. The injections were prepared extemporaneously and administered intraperitoneally (ip), the dose of 0.1 ml/10 g body weight. The dose of DHA and LPS was chosen according to the protocol of Zhao et al.[13]

The animals were divided into three groups (n = 10 in each group), after acclimation for seven days. Group1 (H_2O+H_2O), group2 (LPS+H_2O), group 3 (LPS+DHA). Neuroinflammation was induced by administration of 500 µg/kg ip. of LPS for seven days then treated with DHA (55 mg/kg, per os) for 7 consecutive days, from the 8th to the 14th day.

3. Behavioral tests

3.1. Open-field test

Open-field test was used to analyze the spontaneous exploratory activity and curiosity of the animals when faced with a new and spacious environment. The test was carried out in a lighted room. Normally, an animal tends to spend more time at the periphery of the device rather than in the center, considered the most anxiety-provoking area.[26][27][28][29] Each mouse was placed in the central area and then free to explore the new environment for 10 min.

The variables measured were

– Number of squares crossed (corresponding to the distance traveled);
– The number of adjustments carried out.

Statistical analyzes were processed over the entire duration of the test.

3.2. Raised cross maze

The elevated cross maze is a device shaped like a cross with two opposing open arms (30 cm) and two closed arms (30 cm). In the center was an open platform (5.5cm x 5.5cm) on all four arms. The maze was located 53 cm above the ground and its center was dimly lit. It consists of assessing the behavior of an animal on this device by evaluating the number of entries as well as the time spent in the open arms, parameters of the anxious state.[30]

The animals were placed on the central platform, facing one of the open arms, and they were free to explore the maze for 5 min. Locomotor activity and time spent in the different zones (open arms, closed arms and center) were recorded using a camera. These variables made it possible to calculate the anxiety index of each animal; through the following equation:

$$\text{Anxiety index} = 1 - \left[\frac{\text{time in open arms}}{5\text{min}} + \frac{\text{number of entries in open arms}}{\text{Total entries}}\right]/2$$

The experimental design is described in **Fig.1**.

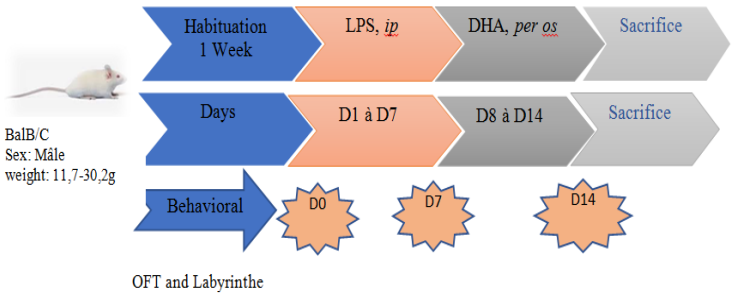

Figure 1: Experimental design. The behavioral tests (OFT and elevated cross maze) were carried out 24 hours before the start of the experiment (D0); then on D7 and D14 of the experiment.

4. Animal sacrifice, tissue preparation and histological evaluation

Twenty-four hours after the end of the behavioral tests (D8 and D15) the mice were anesthetized with isoflurane then decapitated. The brains were then gently removed, washed in a sodium chloride solution (9% NaCl), then post-fixed for 3 weeks in 10% formalin. The samples were embedded in paraffin and then cut sections (1-1.5 mm thick) using a microtome. Finally, the sections were mounted on object slides and then stained with hematoxylin-Eosin (HE). The reading was made using an optical microscope (x100).

5. Statistical analysis

Data were analyzed using Graph Pad Prism 8.4.3 software (Graph Pad Inc., La Jolla, CA, USA). Results were expressed as mean ± standard error (M±SEM). Bivariate analysis using the ANOVA test was performed. Multiple comparisons were carried out using the Tukey, Sidak and Dunnett tests. The results were considered statistically significant for $p<0.05$.

RESULTS

1. Effects of DHA on behavior

1.1. Effect on locomotor activity

The open field test made it possible to evaluate the effects of DHA on locomotor activity (distance traveled). Indeed, we observed a statistically significant difference between groups 1 and 2 (p=0.002) seven days after LPS treatment. A non-significant increase (p=0.34) in this variable was reported observed after the administration of DHA (Fig. 2). Regarding the number of rectifications, the same trend was observed (Fig. 3).

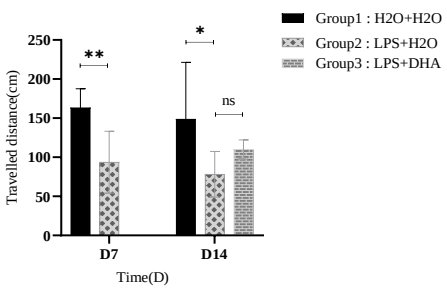

Figure 2: travelled distance, open field test. Two-way ANOVA test, Tukey's multiple comparison test (comparison with different groups). (**): P=0.002; (*): P=0.021; ns: not significant.

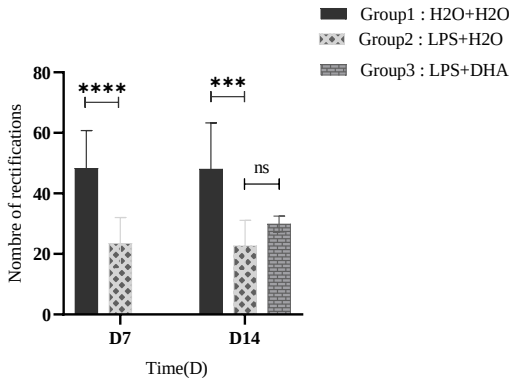

Figure 3: Number of rectifications, open field test. Two-way ANOVA test, Tukey test. (****): P<0.0001; (***): P=0.0002; ns: not significant; (D): Days.

1.2. Effect on anxiety

The effect of DHA on anxiety was evaluated by the elevated plus maze test. Our results do not show a non-significant increase in the anxiety index between groups 2 and 3 after DHA treatment (p=0.99). (Fig. 4).

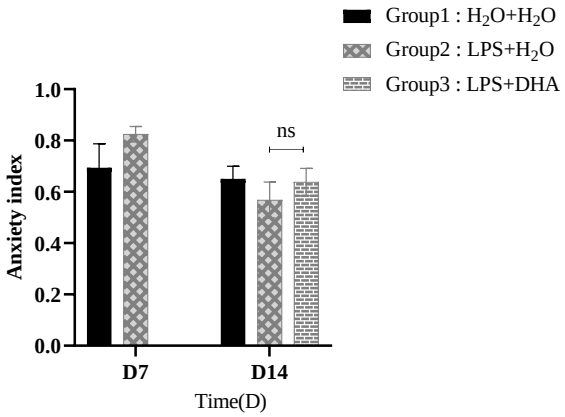

Figure 4: Anxiety index, elevated plus maze test. Two-way ANOVA test followed by Tukey test. ns: not significant; (D): Days.

2. Effect of DHA on histological appearance

Whole brain sections were analyzed under a light microscope for identification of possible tissue damage.

In the control group (group 1), light microscopic examination revealed normal tissue. Control sections contained pyramidal cells with a pale vesicular nucleus and distinct nucleolus, as well as normal glial cells (Fig. 5.a).

Sections from group 2 contained a greater number of pyramidal cells with a pale vesicular nucleus and a prominent nucleolus compared to the control group. Pyramidal cells with dark vesicular core and multiple processes were also observed in group 2. In addition, some basophilic cells were surrounded by empty spaces (apoptotic cells), consistent with intravascular hemorrhage linked to an inflammatory reaction and foci of ischemic necrosis (Fig. 5.b1).

Section sections of the DHA-treated group show neurons and macrophage cells with a pale vesicular nucleus and an obvious nucleolus (as a perinuclear halo). In addition, these sections contained dark, degenerating polymorphonuclear cells with a phagocytic effect indicating possible tissue repair (Fig. 5.c1).

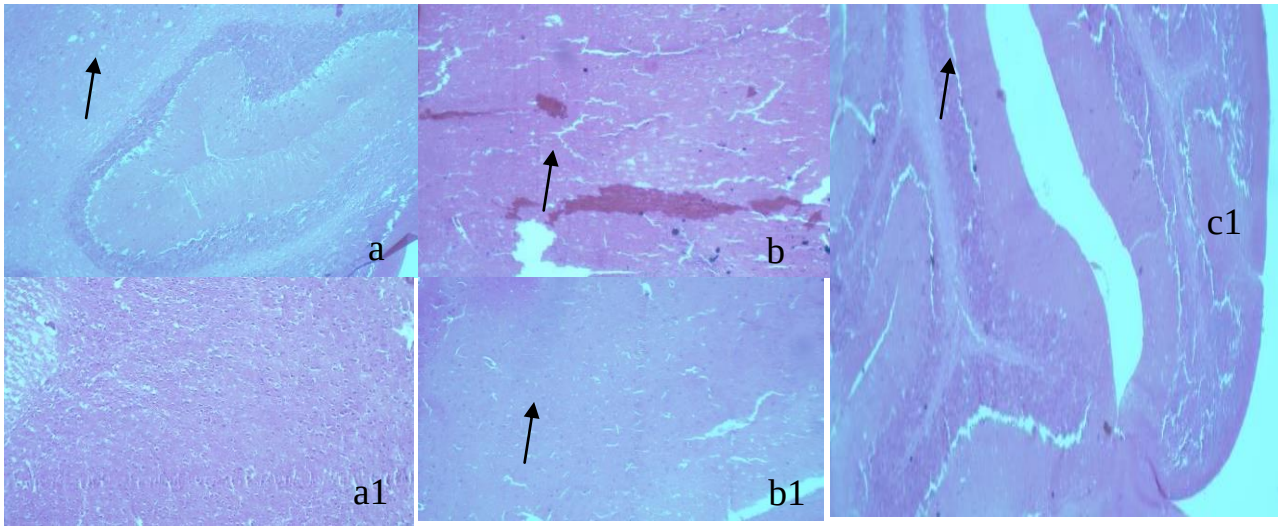

Figure 5: Effect of DHA on the histological profile. On D8: a) Control group (H2O+ H2O); b) Group (LPS+ H2O). D14: a1) Control group (H2O+ H2O); b1) group (LPS+ H2O) c1); group (LPS+ DHA). Gx100.

DISCUSSION

The present work aimed to evaluate the effects of the administration of docosahexaenoic acid on the anxiety-like behavioral profile and cerebral neuronal damage in a model of neuroinflammation.

LPS-induced neuroinflammation is the most widely used model to study the pathophysiological mechanisms involved in neurodegeneration.[31] Indeed, *ip* administration of LPS induces the activation of TLR4 receptors and inflammatory pathways leading to a glial reaction and neuronal loss, which contributes to memory disorders and behavioral changes. Also, acute and chronic inflammation play a role in certain neurodegenerative diseases.[32] Several studies have shown the effectiveness of LPS in inducing neuroinflammation, depending on the dose, method of administration as well as time.[13][16][33][34]

In our study, locomotor activity as well as anxiety state were assessed by open field and elevated maze tests respectively.

Thus, our study the administration of LPS led to a reduction in the distance traveled and the number of straightenings. Other authors have also reported this observation.[35][36][37] The restorative effect of DHA on neuroinflammation has been investigated in several studies.[38][39][40] Our results show a non-significant increase in the distance traveled, the number of straightenings as well as the anxiety index. This could be explained by a possible dose-dependent effect of DHA. The differences in results could be explained by the methodological difference as well as the experimental conditions, such as the dose used as well as the intensity of the lighting during the behavioral tests.[41] This would demonstrate the potentiation of the mouse's anxious state by LPS.

Ip administration of LPS induces inflammatory lesions, as reported in the literature.[15][38][42][43] Our results show an inflammatory-type reaction seven days after LPS administration in group 2 (Figure 5b). A similar profile was observed on brain sections from the water-treated group, seven days after LPS administration. This result suggests non-inflammatory lesions caused by water (figure 5a1). An anti-inflammatory effect was found after DHA treatment, as described in a chronic dextran sulfate sodium exposure model in mice.[40]

CONCLUSION

The effects of DHA were investigated in mice in a model of neuroinflammation induced by LPS. The results of behavioral evaluation and histopathological examination show that peripheral administration of 500 mg/kg DHA exerts weak neuro-reparative effects in a neuroinflammation model. Further study on the optimal dose of DHA. In addition, immunohistochemical studies are necessary to identify the mechanisms involved in the lesions found in the control group; also, receptors involved in this repair mechanism of inflammation-related diseases targeting neuroinflammation.

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