Protective effect of ω-3 fatty acids in a rat focal cerebral ischemia-reperfusion model

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ABSTRACT

Objective: To investigate possible neuroprotective effects of dietary supplementation of fish oil in brain ischemia-reperfusion (I/R).

Methods: This investigation took place in the Experimental Research Unit, Firat University, Elazig, Turkey, from January-February 2006. The study was carried out on 12 male Wistar rats; divided into 2 groups: I/R (control) and I/R + ω-3 essential fatty acids (EFA) (experiment). The rats in the I/R group received only ordinary rat food before middle cerebral artery (MCA) occlusion. The I/R + ω-3 EFA group received omega-3 fatty acid daily via intragastric gavage (300 mg/kg Marincap capsule) with normal food before MCA occlusion for 30 days. Structural alterations in the brain tissues were semi-quantitatively analyzed (0: absent, +: slight, ++: moderate, +++: severe).

Results: There was evident severe (+++) edema, vacuolization, and eosinophilic degeneration in the I/R group, while only slight (+) edema and eosinophilic degeneration in the I/R + ω-3 EFA group in which no vacuolization was determined. These findings are consistent with the available studies in this field.

Conclusion: Results from this study indicate the beneficial effects of ω-3 EFA supplementation in prevention of I/R - induced damage in rats.

Neurosciences 2007; Vol. 12 (3): 198-201

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Received 20th January 2007. Accepted 19th February 2007.

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Cerebral ischemia is one of the leading causes of death in aged populations. Thromboembolic occlusion of the artery is the most important cause of ischemia in patients. There is no proven efficient treatment for this condition. In brain ischemia, cerebral blood flow is reduced in brain regions that are supplied with oxygen by the occluded vessels. In addition to the lack of blood flow and oxygen delivery, the restoration of blood flow has also been reported to contribute to cell damage due to the generation of free radicals.[1,2] Recent studies show that over production of free radicals is important in the pathogenesis of the cerebral damage induced by ischemia-reperfusion (I-R).[2-5] Eicopentaenoic acid (EPA), docosahexaenoic acid (DHA), and ω-linoleic acid (ALA) are known as ω-3 essential fatty acids (ω-3 EFA).[1,6,7] Both EPA and DHA are members of the polyunsaturated fatty acids (PUFA) family and are found abundantly in fish oil, whereas ω-linoleic acid is found in vegetable sources such as soybean and linseed oil.[1,7-10] These fatty acids are important for normal cerebral development and brain function of vertebrates.[1,7,11] Docosahexaenoic acid is involved in memory formation,[12] excitable membrane function,[13] photoreceptor cell biogenesis and function,[14] and neuronal signaling,[8] and has been implicated in neuroprotection.[15-17] According to the epidemiologic studies among non-emigrated Greenlanders, it has been determined that all the 3.5 % deaths took place because of ischemic heart disease. In western countries, this rate is 10 times as much as in Greenlanders. Eskimos' using many sea products and their inclusion of ω-3 attracts the attention of scientists in this field.[18] It is also reported that ω-3 PUFA intake is associated with a decreased risk of cardiovascular disease and ischemic stroke.[19,20] Umemura et al[21] showed that dietary DHA produced antithrombotic effects via inhibition of TXB2 formation in whole blood and caused a reduction in the size of ischemic cerebral lesions in a middle cerebral artery (MCA) thrombosis rat model. It is reported that chronic administration of DHA contributes to protection against neuronal damage in the hippocampal CA1 region and reduced cognitive deficits caused by transient forebrain ischemia.[22] It is also reported that these fatty acids decrease cerebral lipid peroxidase and act as antioxidants in aged rats.[3,23,24] The aim of the current study was to investigate possible neuroprotective effects of dietary supplementation of fish oil in brain I/R.

Methods. *Animals and treatments.* Twelve adult male Wistar rats from the Experimental Animal Center of Firat University, Medical Faculty, weighing 280-320g, comprised the study material. Before the commencement of the study, necessary ethical approval was obtained from the local ethics committee, and this study was conducted from January to February 2006. The animals were maintained under controlled temperature (21±1°C) and controlled light conditions (light 07:00-19:00 hours). Food (standard pellet diet) and tap water were supplied ad libitum. The investigations were carried out in 12 male Wistar rats, divided into 2 groups I/R and I/R+ ω-3 EFA. The control group of rats received only normal food before MCA occlusion. The I/R+ ω-3 EFA group received omega-3 fatty acid daily via intragastric gavage (300 mg/kg Marincap capsule) with normal food before MCA occlusion for 30 days. The Marincap capsule is made up of by EPA (18%) and DHA (12%).

Middle cerebral artery occlusion. Occlusion of the right MCA was performed by a nylon filament as described previously.[2,25] The MCA was occluded for 60 minutes followed by 24 hours perfusion. Briefly, the right common carotid artery was exposed through a midline incision and carefully dissected from the surrounding tissue using a microsurgery technique. The external carotid artery (ECA) was dissected further distally and coagulated along with the occipital and superior thyroid artery branches, which were then divided. The internal carotid artery (ICA) was isolated and carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was ligated close to its origin with a 7-0 silk suture. Next, a 7-0 silk suture was tied loosely around the mobilized ECA stump, and a piece of 4-0 monofilament nylon suture, with its tip rounded by gentle heating, was inserted into the lumen of the right ECA stump and gently advanced via the right ICA to embed into the right anterior cerebral artery so that the right MCA was occluded at its origin. Reperfusion was accomplished by pulling the filament.

Histopathological examination of the brain. At the end of the reperfusion, all rats were sacrificed and the brains were quickly removed, and perfused transcardiacally with cold saline followed by 4% paraformaldehyde in phosphate-buffered saline. The brains were removed from the skull and placed in neutral formalin (10%) and then cut into 2 mm thick coronal slices for routine histopathological examination by light microscopy. Sections (5 µm-thick) from the paraffin–blocks were stained with hematoxylin and eosin. Structural alterations in the brain tissues were determined as semi-quantitative + sign (0: absent, +: slight, ++: moderate, +++: severe).

Results. In this light microscopic study, widespread necrotic areas, red neurons, vacuolization, congestion, and edema were observed in the cerebral cortex in the I/R group. In the I/R+ ω-3 EFA group, findings of ischemia were not widespread and necrotic areas were absent. While edema, vacuolization, and eosinophilic degeneration in the I/R group were severe (+++), in the I/R+ ω-3 EFA group the edema and eosinophilic degeneration were slight (+) and the vacuolization was determined as absent (0). The light microscopic results are shown in **Table 1** and **Figures 1 & 2.**

Table 1 - Light microscopic findings in the brain tissue of group I (I/R) and group II (I/R+ ω-3 EFA) rats (n=6 in each group).

Light microscopic findings	I/R (control)	I/R + ω-3 EFA
Necrotic areas (infarct)	++	0
Eosinophilic degeneration (red neurons)	+++	+
Edema	+++	+
Vacuolization	+++	0
Congestion	++	++

I/R - ischemia-reperfusion, EFA - essential fatty acids, 0 - absent, + - slight, ++ - moderate, +++ - severe

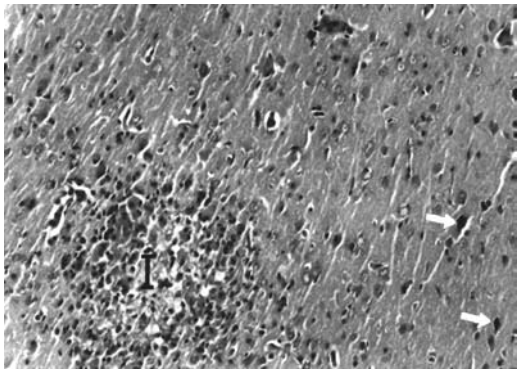

Figure 1 - Ischemia-reperfusion group: red neurons (arrows) and infarct (I) area are seen (Hematoxylin & Eosin x 100).

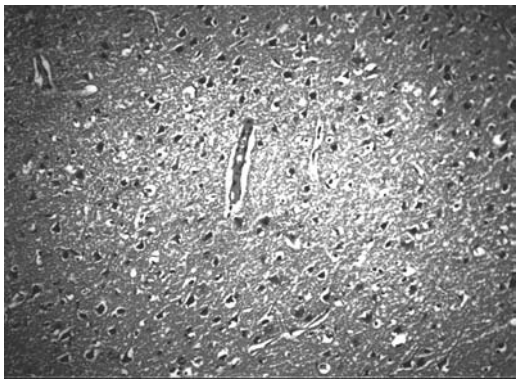

Figure 2 - Ischemia-reperfusion + ω-3 essential fatty acids (experimental) group: Neurons with slight edema and moderate congestion are seen (Hematoxylin & Eosin x 100).

Discussion. Docosahexaenoic acid is involved in memory formation,[12] excitable membrane function,[13] photoreceptor cell biogenesis, and function,[14] neuronal signaling,[8] and has been implicated in neuroprotection.[15-17] It is also reported that ω-3 PUFA intake is associated with a decreased risk of cardiovascular disease and ischemic stroke.[19,20] Kwon et al[3] fed rats with ω-3 (14% menhaden oil ringa) for 6 weeks and the rats that were implemented with I/R were observed to have a decrease in infarct volume when compared with the control group.[3] Similarly, we observed that there was a marked decrease in the study of the brain histopathologic ischemia findings (necrotic area, eosinophilic degeneration, edema, vacuolization, congestion) of the rats fed with Marincap capsules (300 mg/kg) in 30 days analogized with the control group. We found that there was, especially, no infarct area in the I/R + ω-3 EPA group and there was reduction in edema, vacuolization, and eosinophilic degeneration that shows the width of the ischemia finding when compared with the control group (from +++ to +). We think that this reduction happens both with the antioxidants effect of the ω-3 fatty acids,[3] and inhibition of the TXB 2 formation.[21]

Cao et al[1] found that the neuron damage of the hippocampus CA1 region of the animals they gave E-DHA (ethyl all cis-4,7,10,13,16,19-docosahexaenoic acid; 98% pure, 200 mg/kg day) for 10 weeks was less than the control group and the locomotor hyperactivity declined. They said that this effect was due to the speciality of the E-DHA's antioxidants. In our study, we similarly determined that the findings showing the neuron damage reduced in the I/R + ω-3 EFA group compared with control group. Okada et al[22] reported that chronic administration of DHA contributes to protection against neuronal damage in the hippocampal CA1 region and reduced cognitive deficit caused by transient forebrain ischemia. They suggested that administration of DHA decreased the brain AA content, which might be attributed to the protective effect of DHA treatment on neuronal damage.

In our study, we determined that the ω-3 EFA showed a protective feature against transient brain ischemia and the brain histopathologic ischemia findings decreased when they are compared with the control group. These findings show a similarity with the other studies realized in this field.

In conclusion, our results show the beneficial effects of ω-3 EFA in the prevention of I/R-induced damage in rats, and suggest testing ω-3 EFA in further studies for prevention of possible I/R-induced damage. We are of the belief that by using biochemical analyses in new studies, the brain I/R damage's exploration will be suitable.

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