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ScienceDirect
Nutrition Research 28 (2008) 798 – 808
Nutrition Research
www.nrjournal.com

Long-term treatment with fish oil prevents memory impairments but not hippocampal damage in rats subjected to transient, global cerebral ischemia

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Received 23 May 2008; revised 4 September 2008; accepted 11 September 2008

Abstract

Cerebral ischemia leads to neurodegeneration and cognitive impairment. Fish oil (FO) constitutes a rich dietary source of ω-3 polyunsaturated fatty acids especially docosahexaenoic acid (DHA). The objective of the present study was to investigate whether long-term treatment with commercial, high concentration DHA-containing FO could be effective in alleviating both the cognitive and neurodegenerative deficits caused by transient, global cerebral ischemia (TGCI) in rats. Naive rats were trained for 10 days in an 8-arm radial maze task and then subjected to TGCI for 15 minutes (4-VO model) 3 days later (day 13). Retention of the previously acquired cognition (ie, memory) was assessed weekly on days 20, 27, 34, 41, 48, and 55 and measured by 3 behavioral parameters as follows: (i) latency to find the goal box, (ii) number of reference memory errors, and (iii) number of working memory errors. The extent of pyramidal cell death in the hippocampus was examined at the end of the behavioral analysis on day 43. Fish oil (300 mg/kg DHA, gavage) administration occurred once daily beginning 3 days before TGCI (the last day of training) and continued until day 41. Transient, global cerebral ischemia markedly disrupted memory performance measured by all 3 parameters ($P < .0001$ vs sham). This amnesic effect of ischemia persisted until the end of the behavioral analysis. Treatment with FO progressively reversed the TGCI-induced retention deficit until rats achieved control levels. This protective effect of FO on learning/memory function was clearly observed after both daily and cumulative data analysis ($P < .001$-0.01 vs vehicle). Such memory improvements remained statistically significant, even after cessation of FO treatment, indicating a sustained effect of FO. In contrast, FO failed to prevent ischemia-induced hippocampal damage in areas CA1, CA2, or CA4. Therefore, the present findings suggest that long-term FO treatment is able to facilitate functional recovery after ischemic brain damage, an effect that was distinct from hippocampal damage.

Keywords: Global cerebral ischemia; Neurodegeneration; Fish oil; Docosahexaenoic acid; Learning and memory, Rats

Abbreviations: α-LA, α-linolenic acid; ANOVA, analysis of variance; AvRM, aversive radial maze; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; TGCI, transient, global cerebral ischemia; RT, retention trial; 4-VO, 4-vessel occlusion.

1. Introduction

Neuropsychologic dysfunction, including learning and memory impairment, is a major outcome resulting from transient, global cerebral ischemia (TGCI). A postischemia,

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doi:10.1016/j.nutres.2008.09.004

amnesic syndrome has been described in humans who survive cardiopulmonary arrest and comprises a combination of cognitive, executive, sensory, and motor impairments [1-3]. In some circumstances, patients became severely disabled and had a wide range of memory deficits and executive dysfunction that negatively impact psychosocial and vocational reintegration and impose a great burden on a patient's family [4,5]. Although experimental studies have identified many potentially effective neuroprotective agents, no clinically effective, therapeutic strategy has yet been found to treat cerebral ischemia [6]. Therefore, the search for substances that could be safe and effective in the treatment of patients having, or with high-risk factors for, cerebral ischemia remains an urgent priority.

ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA, 22:6n-3) has gained attention in memory and brain functions. Docosahexaenoic acid plays a crucial role in maintaining structural and functional integrity of biological membranes [7-9]. In the brain and retina, DHA comprises more than 20% and 50% of the membranes phospholipids, respectively [10]. In the organism, DHA is synthesized from α-linolenic acid (α-LA, 18:3n-3), an n-3 fatty acid found mainly in green leafy vegetables, flaxseed oil, canola oil, linseed oil, and walnuts. However, the oil extracted from certain fish species constitutes a major dietary source for both DHA and eicosapentaenoic acid (EPA, 20:5n-3) [8,9]. Once incorporated into the phospholipids of the cell membrane, DHA modulates neurotransmitter release, membrane-bound enzyme and ion channel activity, gene expression, and synaptic plasticity [11,12]. Numerous peripheral and central nervous system disorders have been associated with an imbalance in n-6 to n-3 polyunsaturated fatty acids, mainly arachidonic acid and DHA, respectively [8,9,12]. Dietary supplementation with fish oil (FO) containing standardized concentrations of EPA and DHA has been recommended by the American Heart Association for patients with documented chronic heart disease [7].

Evidence indicates that DHA and its exogenous precursor α-LA may be effective in reducing ischemic brain damage when given before ischemia. For example, oral treatment with DHA for 21 days before TGCI reduced both learning/memory deficits and hippocampal cell death in rats [13]. In a gerbil model of TGCI, oral administration of ethyl-DHA for 4 weeks before ischemia reduced the rate of mortality, postischemia brain hypoperfusion, cerebral edema, and arachidonic acid–derived mediators, and improved locomotor activity [14-16]. A robust neuroprotective effect was found when a single dose of either α-LA or DHA was administered intravenously 3 days before ischemia [17]. The beneficial effect of DHA has also been observed in animal models of stroke [18]. In most of these studies, DHA was administered for several days before the ischemic event, suggesting the prophylactic use of dietary supplements. Therefore, the findings suggest that DHA could be a safe, relatively inexpensive, and orally available neuroprotective strategy for preventing or treating cerebral ischemia, especially in populations with high-risk factors. Although TGCI occurs mainly because of unexpected, reversible cardiac arrest, hypoxic/ischemic brain damage also is often likely to occur (eg, during surgical or diagnostic procedures that may reduce or interrupt cerebral blood flow) [19].

However, in the studies mentioned above, only DHA was administered and its putative neuroprotective effect was measured for a few postischemic days [13,18]. Whether oral administration of FO, a principle dietary source of DHA and EPA, also could be effective in preventing the consequences of cerebral ischemia is not well understood. To our knowledge, only one other study has been conducted showing that FO reduced the number of apoptotic cells in the hippocampus, an effect that was measured after a few hours postischemia [20]. In addition, studies that have used behavioral parameters to assess the beneficial effects of DHA in models of cerebral ischemia have been scarce [13], and virtually, no study has been undertaken to examine the potential beneficial effects of FO in preventing ischemia-induced cognitive dysfunction. The long-term use of functional end points is an important requirement when the putative efficacy of agents in providing functional recovery after ischemic brain damage is to be tested [21].

Therefore, the objective of the present study was to investigate whether prolonged treatment with a commercially available, standardized FO preparation is effective in alleviating both the cognitive impairment and hippocampal neurodegeneration caused by TGCI in rats. The specific hypothesis was that DHA treatment may protect the brain from ischemic brain damage, and the use of a standardized, high-grade DHA-containing FO formulation may be a safe, effective, and readily available means of treating or preventing the consequences of cerebral ischemia.

2. Methods and materials

2.1. Animals

A total of 40 young adult, male Wistar rats (inbred strain, 3-4 months of age, 270-300 g body weight) was acquired from the local vivarium of the State University of Maringá (Paraná, Brazil). Nine of these rats underwent sham surgery (controls), and 31 were subjected to TGCI. The rats were housed at a controlled temperature (22°C ± 1°C) on a 12-hour alternating light-dark cycle (lights on at 07:00 hours). The animals had free access to tap water and a standard commercial chow diet containing carbohydrate (660 g/kg), protein (230 g/kg), fat (40 g/kg), fiber (60 g/kg), and vitamins plus minerals (10 g/kg) (Nutrilab-CR1; Nuvital Nutrients, Curitiba, Paraná, Brazil). The experimental procedures used in the present study adhered to the ethical principles of the Brazilian College of Animal Experimentation and were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, Paraná, Brazil.