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Fish oil provides robust and sustained memory recovery after cerebral ischemia: Influence of treatment regimen

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HIGHLIGHTS

- Global cerebral ischemia induces profound memory impairment as well as hippocampal and cortical damage.
- Fish oil (FO) given three days prior to and during the first week of ischemia reverted that deficit.
- This effect was lost when FO treatment was delayed for two weeks after ischemia.
- The antiamnesic effect of FO occurred in the absence of histological neuroprotection.
- An acute, anti-ischemic action of FO may underlie its long-lasting antiamnesic effect.

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ABSTRACT

We previously reported that long-term treatment with fish oil (FO) facilitates memory recovery after transient, global cerebral ischemia (TGCI), despite the presence of severe hippocampal damage. The present study tested whether this antiamnesic effect resulted from an action of FO on behavioral performance itself, or whether it resulted from an anti-ischemic action. Different treatment regimens were used that were distinguished from each other by their initiation or duration with regard to the onset of TGCI and memory assessment. Naive rats were trained in an eight-arm radial maze, subjected to TGCI (4-VO model, 15 min), and tested for memory performance up to 6 weeks after TGCI. Fish oil (docosahexaenoic acid, 300 mg/kg/day) was given orally according to one of the following regimens: regimen 1 (from 3 days prior to ischemia until 4 weeks post-ischemia), regimen 2 (from 3 days prior to ischemia until 1 week post-ischemia), and regimen 3 (from week 2 to week 5 post-ischemia). When administered according to regimens 1 and 2, FO abolished amnesia completely. This effect persisted for at least 5 weeks after discontinuing the treatment. Such an effect did not occur, however, in the group treated according to regimen 3. Hippocampal and cortical damage was not alleviated by FO. The present results demonstrate that FO-mediated memory recovery (or preservation) following TGCI is a reproducible, robust, and long-lasting effect. Moreover, such an effect was found with a relatively short period of treatment, provided it covered the first days prior to and after ischemia. This suggests that FO prevented amnesia by changing some acute, ischemia/reperfusion-triggered process and not by stimulating memory performance on its own.

1. Introduction

Reversible cardiac arrest is a condition that tends to increase as the guidelines for successful cardiopulmonary resuscitation improve worldwide. Resuscitation from cardiac arrest is attempted in approximately 500,000 individuals each year in the United States and Europe, and the successful return of spontaneous circulation can be achieved in 20–50% of those patients, from which only 2–15% will be discharged alive from the hospital. Death occurs mainly because of cardiac or neurological complications within the first days or weeks after cardiac arrest [1,2]. Transient, global cerebral ischemia (TGCI) is an immediate and serious outcome of reversible cardiac arrest. TGCI patients who survive long after cardiac arrest may develop a neuropsychological syndrome that consists of executive/cognitive dysfunction (e.g., attention, working memory, language, and decision-making deficits) and sensory and motor impairments [1,3–6]. In the most severe cases, profound memory impairments (e.g., anterograde and retrograde amnesia) and other executive dysfunctions (e.g., attention deficits, verbal communication disability, temporal confusion, and

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visuo-spatial disorientation) hamper the patient from professionally and socially reintegrating [6,7]. To date, no pharmacological treatment has effectively minimized the functional impairments following cerebral ischemia [8]. Therefore, studies that seek to identify strategies that could be safe and effective in the treatment of patients who have or at high risk for ischemic cerebral disease are needed.

In this scenario, omega − 3 polyunsaturated fatty acids (PUFAs), mainly docosahexaenoic acid (DHA; 22:6n − 3) and eicosapentaenoic acid (EPA; 20:5n − 3), may represent a potential strategy. Disturbances in PUFA metabolism play a role in several diseases, including collagen vascular diseases, hypertension, coronary heart disease, atherosclerosis, diabetes mellitus, neuropsychiatric diseases, and cancer [9,10]. Some evidence suggests that DHA has therapeutic potential against the neurodegenerative and functional impairments caused by global [11] and focal cerebral ischemia [12]. As pure substances, however, neither DHA nor EPA is yet available for clinical use. However, fish oil (FO) is the most important source of DHA/EPA, and standardized, high-grade pharmaceutical FO formulations are easily available for human consumption. For example, the American Heart Association recommends the prescription of FO (Omacor®) for patients with documented chronic heart disease [13]. On this basis, we evaluated whether treatment with a standardized FO formulation (Omega − 3 DHA 250®) effectively prevents the histomorphological and cognitive outcomes of TGCI in rats [14]. In that study, daily FO administration began 3 days prior to TGCI and continued up to 28 days post-ischemia. Under this treatment regimen, FO reversed the amnesic effect of TGCI almost completely, measured in an eight-arm, radial maze task. This beneficial effect of FO on cognition occurred, however, in the absence of histological neuroprotection in the hippocampus. Notably, very similar data were contemporaneously reported by other investigators [15], indicating that the effect of FO in facilitating (or preserving) memory function after TGCI may be a consistent finding. A common methodological feature of these last two studies is the long duration of treatment, which began prior to TGCI and continued to the end of behavioral testing. This implies that FO was administered daily throughout the period of memory assessment. Considering the lack of histological neuroprotection in both Plamondon's and Fernandes's studies, we reasoned that the strong antiamnesic effect of FO could be attributable to a direct action of the active components of FO on some memory-related process that acts far away from and independently of ischemia. Alternatively, FO may exert an enduring and functionally-related anti-ischemic action by interrupting some acute ischemia-triggered pathological steps. Before attempting to ascertain any specific mechanism of action, we approached that question by answering the following: (*i*) Does the effect of FO in facilitating memory recovery is maintained if the treatment is initiated and maintained temporally distant from the onset of ischemia? (*ii*) Does the antiamnesic effect of FO also work after a short treatment duration? (*iii*) Can the antiamnesic efficacy of FO be sustained long after the discontinuation of treatment? Additionally, in a post hoc analysis, we extended the histological analysis beyond the CA1–CA4 subfields of the hippocampus.

2. Material and methods

2.1. Subjects

Male young-adult, Wistar rats (inbred strain, 3–4 months of age, 270–300 g body weight) were used. One hundred four subjects completed the experiments. Of these, 38 rats were assigned to sham operation, and 66 were subjected to TGCI, which were further subdivided into a vehicle-treated group (34 rats) and FO-treated group (32 rats). The rats were housed at a controlled temperature (22 ± 1 °C) on a 12 h alternating light/dark cycle (lights on at 7:00 AM). The animals had free access to tap water and a standard commercial chow diet (Nutrilab-CR1; Nuvital Nutrients, Curitiba, PR, Brazil). The experimental procedures used in the present study adhered to the ethical principles of the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, Paraná, Brazil (authorization no. 015/2008). All efforts were done to minimize the suffering of animals.

2.2. Timeline of FO treatment relative to ischemia and behavioral testing

Fig. 1 shows a schematic of the entire experimental protocol. Naive rats were trained for 10 days (5 days/week) to learn the radial maze task (see Section 2.4 for procedural details). On the last day of training, they were assigned to different groups after balancing for individual learning performance, calculated as the mean latency and mean number of errors recorded during the last 3 days of training (Days 8–10). TGCI was induced on Day 13. The FO- and vehicle-treated groups were divided according to administration regimen 1, 2, or 3, which differed from each other according to the initiation and/or duration of the treatment with regard to the onset of TGCI, retention memory trials (RMTs), and histological analysis. Memory performance was assessed beginning 1 or 2 weeks after TGCI and continued for 5 to 6 weeks, at a rate of one RMT per week (i.e., on Days 20, 27, 34, 41, 48, and 55). One day after the last RMT, the brain was removed and processed for histological analysis.

2.3. Ischemia

TGCI was induced according to the four-vessel occlusion (4-VO) model [16] with modifications. The animals were anesthetized by a mixture of halothane/oxygen (Tanohalo®, Cristália, SP, Brazil) delivered through a mask adapted to the nose. The vaporizer was regulated to the minimal burble flow (0.5 l/min). Under these conditions, the volume of halothane delivered to the animal was further regulated and monitored to maintain the minimal halothane concentration required for efficient anesthesia (evaluated by pinching the animal's tail). The animal was fixed in a stereotaxic frame for bilateral electrocoagulation of the vertebral arteries at the level of the first cervical vertebra. An incision into the ventral neck exposed the common carotid arteries, which were loosely snared with a silk thread. Six hours later, the silk thread was carefully tightened for 15 min in conscious, spontaneously ventilating animals, completing the 4-VO procedure. Throughout occlusion and during the first hour of reperfusion, the rats were maintained in a warming box (inner temperature, 30 ± 1°C) to avoid ischemia-induced brain hypothermia [17]. Loss of the righting reflex within 2 min of carotid occlusion, unresponsiveness to gentle touch, mydriasis, and tonic extension of the paws were considered indicative of effective ischemia. The animals that did not lose the righting reflex or recovered it before the 15-min period were excluded. During surgery, rectal temperature was maintained at 37 ± 0.5 °C with a heating pad and monitored with a digital thermometer (Minipa, APPA MT-520, São Paulo, SP, Brazil) coupled to a rectal probe (Minipa Electronics, Houston, TX, USA) inserted to a depth of approximately 6 cm. Sham-operated animals were subjected to the same surgical intervention, with the exception that both the vertebral and carotid arteries were not occluded.

2.4. Behavioral procedures

Preoperative training and postoperative retrograde memory performance were measured in the aversive, eight-arm radial maze (AvRM) model. Based on the circular platform test [18], the AvRM is a non-food-rewarded task that works on the basis of the rat's natural behavior of avoiding open and illuminated areas and its preference for darkened and enclosed places (in this case, a shelter or goal box located just beneath the opening at the distal end of a given arm). The original concept for the development of the AvRM model was published in 2004 [19] and later improved by changing it from the