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Research report

Fish oil provides a sustained antiamnesic effect after acute, transient forebrain ischemia but not after chronic cerebral hypoperfusion in middle-aged rats

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HIGHLIGHTS

- Transient or chronic brain ischemia causes retrograde amnesia in middle-aged rats.
- Fish oil (FO) given for 9 days after transient ischemia reversed retrograde amnesia.
- The antiamnesic effect of FO persisted for 5 weeks after discontinuing treatment.
- FO failed to prevent retrograde amnesia caused by chronic cerebral hypoperfusion.
- FO serves to treat memory deficit after transient, but not chronic cerebral ischemia.

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ABSTRACT

We reported that fish oil (FO) abolishes retrograde amnesia consistently following transient global cerebral ischemia (TGCI) in young rats, provided it covered the first days prior to and after ischemia. Here, we further evaluated whether FO given post-ischemia in older rats (15–18 months old) is equally effective in facilitating memory recovery. We also tested whether the antiamnesic effect of FO observed after TGCI can be reproduced after chronic cerebral hypoperfusion (CCH). FO (300 mg/kg docosahexaenoic acid [DHA]) was delivered orally 4 h after TGCI and continued once per day for 9 days. In the CCH group, FO treatment began soon after the first stage of 4-VO/ICA and continued daily for 43 days. Two weeks after surgery, the animals were tested for retrograde memory performance across 5 weeks. Both TGCI and CCH caused persistent memory impairment and hippocampal and cortical neurodegeneration. TGCI-induced retrograde amnesia was reversed by FO, an effect that was sustained for at least 5 weeks after discontinuing treatment. In contrast, the memory deficit caused by CCH remained unchanged after FO treatment. Both hippocampal and cortical damage was not alleviated by FO. We conclude that the FO-mediated antiamnesic effect following TGCI can be extended to older rats, even when the treatment begins 4 h postischemia. Such efficacy was not reproduced after CCH. Therefore, the present results support the notion that FO may have therapeutic utility in treating learning/memory dysfunction after acute/transient cerebral ischemia and suggest that such benefits may not apply when a state of chronic cerebrovascular insufficiency is present.

1. Introduction

Ischemic brain disease has an enormous impact on society and leads to a high rate of mortality or renders the survivor temporally or permanently incapacitated. Cerebral ischemia followed by reperfusion may occur acutely following different etiologies and mechanisms. Transient cardiac arrest, severe dysrhythmias,

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and intracerebral arterial occlusion (e.g., thrombotic and embolic) represent the most common causes of global or focal cerebral ischemia [24]. Another condition of cerebrovascular deficiency refers to a state of progressive, chronic cerebral hypoperfusion (CCH), meaning that the amount of blood flow delivered to the brain is insufficient to cope with the needs of metabolizing brain tissue. Chronic cerebral hypoperfusion may be involved in the genesis of chronic, progressive aging-related dementia, including the Alzheimer's dementia type [15]. Preventing or treating some risk factors, mainly hypertension, coronary artery disease, myocardial infarct, dyslipidemia, and atherosclerosis, among others, constitutes the primary recommendation to reduce the prevalence of ischemic brain disease [16]. However, once the individual is afflicted by an acute or chronic hypoxic/ischemic event, a major challenge is preventing brain damage and the consequent functional sequelae. To date, many promising results have been obtained in animal models of cerebral ischemia, but none of these results have been successfully translated to the clinical setting, in part because of safety concerns [39]. Therefore, an urgent need exists to identify alternative strategies that safely and effectively minimize the functional impairments that evolve after brain ischemia.

Some studies indicated that omega-3 polyunsaturated fatty acids, mainly docosahexaenoic acid (DHA; 22:6n-3) [4,5,7,30] and eicosapentaenoic acid (EPA; 20:5n-3) [31], reduce the negative outcome of cerebral ischemia. We have investigated the effects of fish oil (FO) in a rodent model of transient, global cerebral ischemia (TGCI) [1,20]. Fish oil is one of the richest natural sources of omega-3 polyunsaturated fatty acids (PUFAs), mainly DHA and EPA, and it is readily available for human consumption as standardized, high-grade DHA/EPA-containing pharmaceutical formulations. The prescription of FO for patients with documented chronic heart disease is endorsed by the American Heart Association, suggesting that it is safe and beneficial [26]. Our first study showed that persistent retrograde amnesia caused by TGCI in young rats was abolished by FO given daily for 32 days when the treatment began 3 days prior to ischemia. In that study, the antiamnesic effect of FO was sustained for at least 2 weeks after the cessation of treatment [20]. We subsequently demonstrated that FO still afforded a robust antiamnesic effect when the treatment duration was reduced from 32 to 10 days, provided it covered the first days prior to and after ischemia. Moreover, this effect was sustained for at least 5 weeks after discontinuing treatment [1]. The beneficial effect of FO against TGCI-induced cognitive impairment has also been reported by others [34]. The acute and parenteral administration of an n-3 fish oil-based lipid emulsion was found to reduce infarct size in a mouse model of perinatal hypoxia/ischemia [45]. Altogether, these data suggest that FO might be beneficial for the treatment of cerebral ischemia in humans. However, additional studies in animal models are required before FO can be entered into clinical trials.

The studies cited above used young rats, and the treatment was initiated before ischemia. We sought to determine whether the antiamnesic effect of FO observed in young rats can be reproduced in older rats subjected to the same experimental conditions. In the field of neuroprotection, aging is an important variable. Ischemic brain diseases are much more prevalent in aged people. To our knowledge, no study has reported the effects of FO after cerebral ischemia in middle-aged rats. Additionally, unknown is whether FO is effective when treatment begins post-ischemia. Determining clinically relevant treatment time windows would be very beneficial because global and focal cerebral ischemia occurs abruptly, and the first medical care is delayed for hours in the majority of cases [39]. Moreover, the studies mentioned above demonstrate the beneficial effects of DHA and FO in models of acute, transient cerebral ischemia. Whether FO can alleviate the outcome of chronic cerebral hypoxia/ischemia has been scarcely reported [44]. Therefore, in a continuation of our ongoing studies, we evaluated whether the antiamnesic effect of FO observed consistently after TGCI in young rats can be reproduced in middle-aged rats when the treatment begins after ischemia. We also tested whether FO effectively mitigates memory impairment caused by CCH in middle-aged rats [21,33].

2. Material and methods

2.1. Subjects

A total of 102 male, middle-aged Wistar rats (12 to 15 months of age) were acquired from our local vivarium. Of these, 51 rats were assigned to the TGCI experiment, and the remaining rats were assigned to the CCH experiment. Prior to any experimental manipulations, the rats were acclimated to the laboratory vivarium for 1–2 weeks and maintained under a controlled temperature ($22 \pm 1\,^{\circ}C$) on a 12 h/12 h alternating light/dark cycle (lights on at 7:00 AM) throughout the study. 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 (CEEA) of the State University of Maringá, Paraná, Brazil (authorization no. 044/2008).

2.2. Surgeries

The surgical procedures were performed as described previously for TGCI [1,20] and CCH [21,22,33], with the exception of modification of the anesthesia procedure in the CCH groups. Briefly, halothane/oxygen anesthesia was delivered through a universal vaporizer to a face mask affixed to the rat's nose. For TGCI, the vertebral arteries (VAs) were bilaterally electrocoagulated, followed by transient occlusion (15 min) of the common carotid arteries (CCAs) 5–6 h later by carefully tightening a silk thread that passed around the CCA (i.e., the 4-VO model with modification) [35]. Chronic cerebral hypoperfusion was provoked by bilaterally electrocoagulating the VAs, followed by permanent, stepwise ligation of the internal carotid arteries (ICAs) according to the sequence VA → ICA → ICA, with an interstage interval (ISI, →) of 4 days (i.e., permanent, 3-stage 4-VO/ICA model of CCH) [3,21,33]. For bilateral occlusion of the VA, the tip of a unipolar electrode was inserted into the alar foramen of the first cervical vertebrae and gently rotated until the presence of hemorrhage ensured irreversible vessel rupture. The hemorrhage was then immediately staunched by an electrical current of 3–4 mA. The ICAs were carefully dissected from adjacent tissues and permanently occluded using cotton thread. After each occlusion stage, the incision was sutured, and the animal was returned to its home cage until the next surgery. Throughout occlusion and during the first hour of reperfusion after TGCI or for 1 h after each vessel occlusion stage in the case of 4-VO/ICA, the rats were maintained in a warming box (inner temperature set to $30 \pm 1\,^{\circ}C$) to avoid hypoxia/ischemia- or anesthesia-induced brain hypothermia [36]. During surgery, rectal temperature was maintained at $37 \pm 0.5\,^{\circ}C$ with a heating pad and monitored with a digital thermometer (Minipa, APPA MT-520, São Paulo, São Paulo, 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 the VAs and CCAs were not occluded.

2.3. Experimental protocol

Fig. 1 schematically illustrates the timeline of FO treatment relative to the period of TGCI, CCH, memory assessment, and