Available online at www.sciencedirect.com
SCIENCE DIRECT
Life Sciences 78 (2005) 74 – 81
Life Sciences
www.elsevier.com/locate/lifescie

Chronic administration of ethyl docosahexaenoate decreases mortality and cerebral edema in ischemic gerbils

Dehua Cao [a], Mei Li [a], Renhao Xue [a], Weifa Zheng [b], Zhili Liu [a,*], Xiaomei Wang [a]

[a] *Department of Biology, Nanjing University, 22 Hankou Road, Jiangsu 210093, PR China*
[b] *Key Laboratory of Medical Plant, Xuzhou Normal University, Tongshan, Jiangsu 221116, PR China*

Received 20 April 2004; accepted 8 April 2005

Abstract

Dietary docosahexaenoic acid (DHA) intake can decrease the level of membrane arachidonic acid (AA), which is liberated during cerebral ischemia and implicated in the pathogenesis of brain damage. Therefore, in the present study, we investigated the effects of chronic ethyl docosahexaenoate (E-DHA) administration on mortality and cerebral edema induced by transient forebrain ischemia in gerbils. Male Mongolian gerbils were orally pretreated with either E-DHA (100, 150 mg/kg) or vehicle, once a day, for 4 weeks and were subjected to transient forebrain ischemia by bilateral common carotid occlusion for 30 min. The content of brain lipid AA at the termination of treatment, the survival ratio, change of regional cerebral blood flow (rCBF), brain free AA level, thromboxane B_2 (TXB_2) production and cerebral edema formation following ischemia and reperfusion were evaluated. E-DHA (150 mg/kg) pretreatment significantly increased survival ratio, prevented post-ischemic hypoperfusion and attenuated cerebral edema after reperfusion compared with vehicle, which was well associated with the reduced levels of AA and TXB_2 in the E-DHA treated brain. These data suggest that the effects of E-DHA pretreatment on ischemic mortality and cerebral edema could be due to reduction of free AA liberation and accumulation, and its metabolite synthesis after ischemia and reperfusion by decreasing the content of membrane AA.

Keywords: Ethyl docosahexaenoate; Arachidonic acid; Transient forebrain ischemia; Survival ratio; Cerebral edema; Regional cerebral blood flow; Gerbil

Introduction

Cerebral ischemia or stroke, one leading cause of death and long-term disability in aged populations, often results in irreversible brain damage and subsequent loss of neuronal function. There is no proven efficient treatment for this condition, primarily because the pathophysiology involved is not yet well understood (Read et al., 1999; Parnham and Sies, 2000). It is well known that ischemic insults to brain induce a rapid increase in the levels of free fatty acids, which are liberated from membrane phospholipids by the action of phospholipase during ischemia and consist mainly of arachidonic acid (AA, 20:4n-6) (Rehancrona et al., 1982; Ikeda et al., 1986; Yoshida et al., 1986; Kubota et al., 1998). The liberated free AA after ischemia is generally thought either to be reincorporated into the membrane phospholipids (Yoshida et al., 1986; Kubota et al., 1998; Pilitsis et al., 2002), or to be as the precursor of prostaglandins (PGs), leukotrienes (LTs), thromboxanes (TXs) and their bioactive eicosanoids (Siesjo et al., 1995; Mrsic et al., 2002), several of which, such as prostaglandin I_2 (PG I_2) and thromboxane A_2 (TXA_2), have been implicated in the edema formation and neuronal injury following ischemia (Gaudet et al., 1980; Bhakoo et al., 1984; White et al., 2000). Therefore, attenuation of AA liberation and subsequent eicosanoid production could have a neuroprotective benefit in the treatment of stroke.

Dietary marine oils rich in n-3 fatty acids, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA,

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; E-DHA, ethyl docosahexaenoate; EPA, eicosapentaenoic acid; FAMEs, fatty acid methyl esters; LT, leukotriene; MABP, mean arterial blood pressure; NO, nitric oxide; rCBF, regional cerebral blood flow; PG, prostaglandin; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PGI_2, prostaglandin I_2; PGI_3, prostaglandin I_3; PLA_2, phospholipase A_2; TLC, thin-layer chromatograph; TX, thromboxane; TXA_2, thromboxane A_2; TXA_3, thromboxane A_3; TXB_2, thromboxane B_2.

* Corresponding author. Tel.: +86 25 8359 7401.
E-mail address: liuzl@nju.edu.cn (Z. Liu).

0024-3205/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2005.04.083

20:5n-3), can decrease membrane AA content as n-3 fatty acids compete with AA for esterification into cellular phospholipids and eicosanoid synthesis, favoring the formation of metabolites of the 3 series, which are believed to exert protective effects (Philbrick et al., 1987; Bourre et al., 1990; Minami et al., 1997). Kalman et al. (1992) reported that dietary marine fish oil causes both a reciprocal replacement of n-6 fatty acids with n-3 fatty acids and a decreased formation of the cyclooxygenase and lipoxygenase products of the arachidonate cascade in the brain capillary endothelial cells in rats. Recently, Umemura et al. (1995) showed that dietary DHA produced antithrombotic effects via inhibiting the formation of thromboxane B_2 (TXB_2), the stable degradation of TXA_2, and caused a reduction in the size of ischemic cerebral lesions in a middle cerebral artery thrombosis rat model. Furthermore, Okada et al. (1996) 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. However, the effects of DHA pretreatment on the ischemic insult induced changes of free arachidonic acid (AA) levels, which are correlated with the pathogenesis of cerebral ischemia, were not studied.

The aim of this study, therefore, is to investigate the effects of chronic administration of ethyl docosahexaenoate (E-DHA), the esterified DHA more effectively to be absorbed and incorporated into tissues than its free form (Krokan et al., 1993), on mortality and cerebral edema formation by examining the survival ratio, change of regional cerebral blood flow (rCBF), brain free AA level, TXB_2 production, and cerebral water content following transient cerebral ischemia induced by bilateral carotid occlusion in the Mongolian gerbil, an animal that lacks normal connections between the carotid and vertebrobasilar system and has been used as a model for cerebral ischemia and infarction by easily occluding the common carotid arteries at the neck that makes ischemia-induced morphological changes similar to what occurred in other experimental animals (Kirino, 1982), which allows us to probe in vivo whether or not the E-DHA pretreatment for protective action against cerebral ischemia could be associated with the reduction of free AA liberation and accumulation, and its metabolite synthesis.

Methods

Animals

Adult male Mongolian gerbils (Experimental Animal Center of Zhejiang Medical University, China), weighing 50–70 g, were used for all the experiments. Before and after ischemia, they were housed, six in a cage, at the constant room temperature of 21–22 °C under a light cycle of 12/12 h (7:00 a.m./7:00 p.m.). The animals were allowed free access to food and drinking water. The adaptation and experiments were performed in strict compliance with the internationally accepted principles and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Treatments

Gerbils were administrated E-DHA (98% pure, Harima Chemicals Co. Ltd., Japan), emulsified in 5% gum Arabic solution, with a dose of 100, 150 mg/kg (1 ml/kg) or with a similar volume of vehicle through oral route, once a day, for a period of 4 weeks before ischemic procedure started.

Fatty acid analysis of brain lipids

Cerebral hemispheres were quickly removed from experimental animals at the termination of pretreatment, frozen in liquid nitrogen, and stored at −80 °C until fatty acid analysis. The frozen tissues were weighed and homogenized in a chloroform/methanol (2:1, v/v) mixture for lipid extraction (Bligh and Dyer, 1959). The solvent mixture was evaporated to a known volume under nitrogen and the fatty acids were converted to their fatty acid methyl esters (FAMEs) by acid-methanolysis with BF_3-methanol (Sigma) at 60 °C for 1 h. FAMEs were analyzed by a gas–liquid chromatograph (HP 5890; Hewlett-Packard, Avondale, PA, USA) equipped with a flame-ionization detector and a silica capillary column (30 cm × 0.32 mm i.d., SP-2330, Supelco, Bellefonte, PA, USA). The oven temperature was programmed to raise from 170 to 240 °C, and detector temperature was set at 270 °C. Identification of the fatty acids was made by comparison of retention times with those of known standards run under the same conditions. Peak areas were calculated with a Hewlett-Packard HP3396 series II integrator, and the fatty acid concentrations were reported as percent of total fatty acid content.

Surgery

Under 2% halothane anesthesia mixed with 30% O_2 and 70% N_2O, a midline ventral incision was made in gerbil neck and the trachea was cannulated with PE-10 polyethylene catheter. A PE-10 polyethylene catheter was inserted into left femoral artery to monitor arterial blood pressure and to obtain an arterial blood sample for blood gas analysis (the removed blood volume was replaced with saline to avoid hypovolemia). Transient forebrain ischemia was produced by clipping both the right and left common carotid arteries with atraumatic aneurysm clips for 30 min. Following the occlusion, the clips were then removed to restore the blood flow. The same surgical operated animals without carotid occlusion were served as sham animals. During the occlusion and postoperative period, rectal temperature was maintained at 37–38 °C with a thermostatically regulated heating lamp. Halothane anesthesia was turned off immediately after cerebral reperfusion except for the regional cerebral blood flow (rCBF) measure study.

As an experimental protocol, we set up three treatment groups: (1) Sham (sham + vehicle); (2) Vehicle (ischemia/reperfusion + vehicle); (3) E-DHA (ischemia/reperfusion + vehicle + E-DHA). In each treatment group, animals were divided into six subgroups to examine (1) physiological parameters, (2)