Epilepsy Research (2013) **105**, 69—76

journal homepage: www.elsevier.com/locate/epilepsyres

Fish oil attenuates methylmalonate-induced seizures

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Received 5 April 2012; received in revised form 21 December 2012; accepted 3 January 2013
Available online 1 February 2013

KEYWORDS
Seizure;
Methylmalonate;
Fatty acid;
Fish oil;
Oleic acid

Summary Methylmalonic acidemias are inherited metabolic disorders characterized by methylmalonate (MMA) accumulation and neurological dysfunction, including seizures. Dietary fatty acids are known as an important energy source and reduce seizure activity in selected acute animal models. This study investigated whether chronic treatment with fish oil or with oleic acid attenuates MMA-induced seizures and whether maintenance of Na^+,K^+-ATPase activity was involved in such an effect. Adult male Wistar rats were given fish oil (85 mg/kg), oleic acid (85 mg/kg) or vehicle (0.42% aqueous Cremophor EL™, 4 mL/kg/body weight/day), p.o., for 75 days. On the 73th day a cannula was implanted in the right lateral ventricle with electrodes over the parietal cortex for EEG recording. On the 76th day the animals were injected with NaCl (2.5 μmol/2.5 μL, i.c.v.), or with MMA (2.5 μmol/2.5 μL, i.c.v.), and seizure activity was measured by electroencephagraphic (EEG) recording with concomitant behavior monitoring. The effect of prostaglandin E_2 (PGE_2) on Na^+,K^+-ATPase activity of slices of cerebral cortex from NaCl-injected animals was determined. Fish oil increased the latency to MMA-induced tonic-clonic seizures, reduced the mean amplitude of ictal EEG recordings, and prevented PGE_2-induced decrease of Na^+,K^+-ATPase activity in cortical slices *in vitro*. Oleic acid decreased mean amplitude of ictal EEG recordings. The results support that fish oil decreases MMA-induced

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0920-1211/$ — see front matter
http://dx.doi.org/10.1016/j.eplepsyres.2013.01.005

seizures. The decreased sensitivity of Na^+,K^+-ATPase to the inhibitory effect of PGE_2in fish oil-treated animals may be related to the currently reported anticonvulsant activity.

Introduction

Methylmalonic acidemias are inherited metabolic disorders characterized by accumulation of MMA in tissue and body fluids, due to a deficiency of the enzyme methylmalonil-CoA mutase (EC 5.4.99.2) or defects in the synthesis of 5-deoxyadenosylcobalamin, its cofactor. Patients with methylmalonic acidaemia usually present acute encephalopathy, ketoacidosis, developmental and mental retardation and severe neurological dysfunction, including seizures. The mainstay of treatment consists in vitamin B_{12} supplementation for those patients with cofactor deficiency and, low-protein, high-energy diet with carnitine supplementation for vitamin B_{12}-unresponsive patients (Fenton and Rosenblatt, 2001). Methylmalonic acid-induced seizures likely involve primary impairment of mitochondrial function due to disruption of oxidative metabolism (Dutra et al., 1993; Toyoshima et al., 1995; de Mello et al., 1996; Fleck et al., 2004; Maciel et al., 2004) and secondary activation of NMDA receptor-mediated mechanisms (de Mello et al., 1996) with subsequent glutamate decarboxylase inhibition (Malfatti et al., 2003) and production of oxidative (Fighera et al., 1999; Marisco et al., 2003) and nitrosative species (Furian et al., 2007, 2008). Recent evidence also suggests that inflammation plays a role in MMA-induced seizures, since they are attenuated by the COX-2 inhibitor celecoxib (Salvadori et al., 2012).

Methylmalonic acid-induced seizures are associated with a decrease of Na^+,K^+-ATPase activity in the striatum and cerebral cortex of rats and mice (Malfatti et al., 2003; Royes et al., 2007; Ribeiro et al., 2009). Moreover, Na^+,K^+-ATPase activity inhibition highly correlates with total time spent in MMA-induced seizures (Furian et al., 2007; Royes et al., 2006), suggesting that Na^+,K^+-ATPase activity inhibition may be, at least in part, causally related to MMA-induced seizures.

Dietary fatty acids are a key energy source and structural components of membranes. Some fatty acids act as receptor ligands, second messengers and immunomodulators, and therefore play a role in modulating cognitive, neurophysiological and behavioral function (Yehuda et al., 1999).

Fish oil is a common source of n-3 polyunsaturated fatty acids (PUFA), being particularly rich in eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) (Duda et al., 2007). Esterified DHA is the most abundant n-3 PUFA in the brain (Dyall and Michael-Titus, 2008), being particularly important for the structural integrity of neuronal and non-neuronal membranes (Bourre et al., 1991). Fish oil has cardiac antiarrhythmic properties and decreases the risk for cardiovascular events, ischemic heart disease and sudden death (Leaf, 2001; von Schacky and Harris, 2007; Lee et al., 2009). In addition, PUFAs have significant anti-inflammatory (Mori and Beilin, 2004; Calder, 2006) and anti-thrombotic effects (Engstrom et al., 2001), through the modulation of prostaglandin, leukotriene and cytokine pathways.

Chronic treatment of epileptic animals with fish oil decreases neuronal death in the CA1 and CA3 subfields of rats treated with pilocarpine (Ferrari et al., 2008). However, it has also been reported that DHA and EPA supplementation in the diet does not change the threshold for fluorothyl-, PTZ-, kainic acid-, or 6 Hz current-induced convulsions in mice (Willis et al., 2009). Beneficial (Schlanger et al., 2002), minimal transient beneficial (Yuen et al., 2005) or no effect (Bromfield et al., 2008) of n-3 PUFAs supplementation on seizure control in epileptic patients has been reported. Notwithstanding, n-3 PUFAs may directly influence neuronal activity, since a deficiency of these fatty acids alters cell homeostasis by decreasing Na^+,K^+-ATPase activity (Gerbi et al., 1999). Although accumulating evidence suggests that antioxidant and anti-inflammatory treatment (Salvadori et al., 2012) decrease MMA-induced seizures, no study has addressed whether dietary supplementation with fish oil and oleic acid alters the seizures induced by MMA and whether Na+,K+-ATPase is involved in such an effect.

Experimental procedures

Animals and reagents

Forty-eight adult male Wistar rats, weighing on average 201 ± 26.2 g at the beginning and 330 ± 32.7 g at the end of the experimental period, were used. Animals were maintained under controlled light and environment (12:12 h light—dark cycle, $24 \pm 1\,^{\circ}C$, 55% relative humidity) with free access to food (Supra™, Santa Maria, Brazil) and water. The macronutrient composition of the supplied diet was: 60% carbohydrate, 22% protein and 4.5% total fat. Food pellets were analyzed by gas chromatography to determine fatty acid composition. All experimental protocols were designed aiming to keep the number of animals used to a minimum, as well as their suffering. These were conducted in accordance with national and international guidelines of Brazilian Council of Animal Experimentation — CONCEA — and of U.S. National Institute of Health Guide for the Care and Use of Laboratory Animals — NIH Publications No. 80-23, revised 1996, and with the approval of the Ethics Committee for Animal Research of the Federal University of Santa Maria.

Fish oil was extracted from commercially available capsules (Proepa™, Aché Laboratories, Brazil) and analyzed by gas chromatography. Oleic acid (free form) was used as a treatment control and obtained from Synth (Sao Paulo, Brazil), and Cremophor EL™ was purchased from Basf (Germany). All the other reagents were purchased from Sigma (St. Louis, MO, USA).

Gas chromatography analysis

Samples were saponified in methanolic KOH solution and esterified in methanolic H_2SO_4 solution (Hartman and Lago, 1973). Methylated fatty acids were analyzed using an