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Research article

A role for peroxisome proliferator-activated receptor α in anticonvulsant activity of docosahexaenoic acid against seizures induced by pentylenetetrazole

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ABSTRACT

Docosahexaenoic acid (DHA) is the most bioactive fatty acid in the brain with well-known biological effects. Peroxisome proliferator-activated receptors (PPARs) underlie some therapeutic effects of DHA such as anti-inflammation, anti-apoptosis and immune regulation. We investigated probable involvement of PPARα in the anticonvulsant effect of DHA in pentylenetetrazole (PTZ) model of clonic seizures. DHA alone or along with the PPARα antagonist GW6471 were administered to mice by intracerebroventricular (*i.c.v.*) and/or intraperitoneal (*i.p.*) route. The incidence as well as the threshold of clonic seizures was determined by *i.p.* and intravenous infusion of PTZ, respectively. DHA, 0.3 mM inhibited the occurrence of seizures (6 out of 10 mice were protected compared to 0 out of 10 in control group, $p < 0.01$). The seizure threshold (mg/kg) in control group (43.3 ± 2.4) increased to 54.5 ± 2.8, by DHA 0.3 mM ($n = 10$, $p < 0.01$). GW6471 (1 mg/kg, *i.p.,* or 4 and 10 μg/mouse, *i.c.v.*) prevented the anticonvulsant effect of DHA and the increase in seizure threshold, in a dose-dependent manner. GW6471 by itself had no effect on the threshold and the incidence of clonic seizures. PPARα is involved in the anticonvulsant effect of DHA in PTZ model of clonic seizures in mice.

1. Introduction

Docosahexaenoic acid (DHA) is long-chain omega-3 (n-3) polyunsaturated fatty acid (PUFA) derived from α-linolenic acid. Antiepileptic potential of n-3 PUFAs has been extensively investigated by many basic and clinical studies [9,30]. DHA is the most bioactive fatty acid in the brain and seems to be a key element in the observed anticonvulsant action of n-3 PUFAs [30]. DHA inhibits pentylenetetrazole (PTZ)-induced seizures as well as generalized kindled seizures, and also potentiates anticonvulsant activity of some antiepileptic drugs [12,23,31,32]. Moreover, DHA removes "inherent resistance" of 6-Hz seizures to phenytoin and lamotrigine, and prevents development of pharmacodynamic tolerance to lamotrigine in lamotrigine-resistant kindled rats [20]. Some mechanisms are suggested for the anticonvulsant effect of DHA including regulation of physicochemical properties of neural membrane by increase in the membrane fluidity and permeability [24], modifying the voltage-gated ion channels [10], and modulation of synaptic activity and expression of receptors such as glutamate, GABA, dopamine and serotonin [33]. In addition to these mechanisms, there are strong evidences indicating interaction of DHA with peroxisome proliferator-activated receptor (PPAR), so that activation of PPARα and PPARγ by DHA and its metabolites play a major role in the therapeutic effects of DHA such as anti-inflammation, anti-apoptosis and immune regulation [4,11,22,36]. Interestingly, PPARα and γ agonists have been protective against seizures in animal models including PTZ, pilocarpine, nicotine, flurothyl, genetically absence, and audiogenic seizure models [1,2,6,7,25,26,28,29] and epileptic patients [27].

Therefore, the present study was designed to verify possible involvement of PPARα in the protective effect of DHA against seizures induced by PTZ in mice.

2. Materials and methods

2.1. Animals

Adult male NMRI mice (20–25 g, Pasteur Institute of Iran, $n = 200$) were used in this study. The animals were housed in standard polypropylene cages in a room with controlled temperature (23 ± 2.0 °C) and 12 h light/dark cycle (6:00–18:00). They were fed ad libitum with

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rodent's chow and free access to drinking water. Animals were randomly divided to different experimental groups. All experiments were conducted during light phase according to guidelines of Institutional Animals Ethics Committee of Pasteur Institute of Iran (Authorization code 93-0201-13085, 12 January 2015) and European Communities Council Directive of 24 November 1986 (86/609/EEC) in a way to minimize the number of animals and suffering.

2.2. Drugs and administration

In order to ensure DHA completely reaches the brain after a single dose administration, DHA was injected to animals by intracerebroventricluar (i.c.v.) route. For this purpose, an ultrapure DHA (D-2534, ≥98%) was purchased from Sigma-Aldrich. It was mixed with 40% hydroxypropyl β-cyclodextrin, HPB, (Sigma-Aldrich) prepared in distilled water. The mixture was then diluted in artificial cerebrospinal fluid (aCSF) with composition (in mM) of 124.0 NaCl, 25 NaHCO3, 10 D-glucose, 4.4 KCl, 2 MgSO4, 1.25 KH2PO4, and 2 CaCl2 (pH 7.2–7.4). Final concentration of HPB was 10%.

The PPARα antagonist GW6471 (Sigma-Aldrich) was dissolved in dimethylsulfoxide (DMSO, Merck) and injected by intraperitoneal (*i.p.*) or *i.c.v.* route. The volume of *i.p.* injection was 0.1 ml/10 g of mice body weight. The volume of *i.c.v.* injection in all experimental groups was 10 µl/mice except for co-administration of the antagonist and DHA in which the mice first received 2 µl antagonist (or its vehicle) and after 15 min 10 µl DHA (or its vehicle) was injected (12 µl in sum).

The injection into the right cerebral ventricle (10 µl during 30 s) was performed according to the previously-described method [12,15]. Mice were gently restrained by hand to insert a 27-gauge needle (fitted to a 10-µL Hamilton microsyringe) into the skull. Site of injection was 2 mm lateral to the midline, on a line drawn through the anterior base of the ears, and 3.5 mm in depth from the surface of skull skin. After injection, the needle was kept in place for an additional 30 s before it was withdrawn. Mice were then released.

PTZ 60 mg/kg was injected to mice *i.p.* to induce generalized clonic seizure (GC). GC was characterized by clonus of all four limbs with transient loss of righting reflex. If no GC occurred during a 30 min period of observation, the animals were considered protected. Furthermore, the latency to GC occurrence was also recorded.

In order to determine the seizure threshold of each animal, a 30 gauge dental needle, which was connected by a polyethylene tube to a Hamilton microsyringe was inserted into lateral tail vein of restrained mouse. After fixing the needle by adhesive tape, mice were released into a Plexiglas cage and PTZ infusion started. The freely moving mice were received PTZ 10 mg/ml intravenously at a constant rate of 100 µl/min. The mg of PTZ per kg of mice body weight was calculated and considered as the seizure threshold of the animal. The maximum volume of intravenous (*i.v.*) infusion to each mouse was 200 µl. If a mouse did not show GC up to 200 µl infusion, it was excluded from the study.

2.3. Experimental design

2.3.1. PTZ seizure incidence

Nine different experimental groups were considered with 10 mice in each. **Groups 1 and 2** received DHA (0.3 mM) or its vehicle (as control), *i.c.v.* After 15 min, PTZ was injected (60 mg/kg, *i.p.*) to mice and GC incidence was recorded. **Groups 3 and 4** received GW6471 (1 mg/kg) or its vehicle (as control), *i.p.* After 4 h, PTZ was injected (60 mg/kg, *i.p.*) to mice and GC incidence was recorded. **Groups 5–7** received GW6471 (0.1, 0.3, or 1 mg/kg, *i.p.*). After 4 h, mice were given DHA (0.3 mM, *i.c.v.*). After 15 min, PTZ 60 mg/kg was injected *i.p.* and GC incidence was recorded. **Group 8** (control) received GW6471 vehicle (2 µl, *i.c.v.*). After 4 h, DHA vehicle (10 µl, *i.c.v.*) was injected. After 15 min, PTZ (60 mg/kg, *i.p.*) was injected to mice and GC incidence was recorded. **Group9** (control) did not receive any drug or vehicle, and just GC incidence was determined after *i.p.* injection of PTZ.

2.3.2. PTZ seizure threshold

Eleven different experimental groups were considered with 10 mice in each. **Groups 1–4** received DHA (0.1, 0.3, or 1 mM, *i.c.v.*), or its vehicle (10 µl, i.c.v., as control). After 15 min, the seizure threshold was determined in mice by *i.v.* infusion of PTZ. **Groups 5–7** received GW6471 (4 or 10 µg/mouse, *i.c.v.*) or its vehicle (2 µl, i.c.v., as control), and after 15 min, the seizure threshold was determined in mice. **Groups 8, and 9** received GW6471 (4 or 10 µg/mouse, *i.c.v.*). After 15 min, DHA 0.3 mM was injected to mice *i.c.v.* and 15 min later seizure threshold was determined. **Group 10** (control) received GW6471 vehicle (2 µl, *i.c.v.*). After 4 h, DHA vehicle (10 µl, *i.c.v.*) was injected to mice, and 15 min later the seizure threshold was determined. **Group 11** (control) did not receive any drug or vehicle, and just the PTZ seizure threshold was determined.

The dose and time intervals for DHA and GW6471 injections were selected based on the previous studies [7,12,24].

2.4. Statistical analysis

SPSS for Windows software version 16.0 was used for statistical analysis. The data obtained from *i.p.* PTZ test, are expressed as number of mice showed GC to total mice, and analyzed by Fisher's exact test. Data regarding the seizure latency in *i.p.* PTZ test and the seizure threshold in *i.v.* PTZ test are presented as Mean ± SEM and analyzed by one-way ANOVA and Tukey's post hoc test. In all experiments $p < 0.05$ was considered statistically significant.

3. Results

3.1. GW6471 inhibits anticonvulsant effect of DHA in i.p. PTZ test

DHA 0.3 mM significantly inhibited the occurrence of GC in mice ($p < 0.01$). GW6471 1 mg/kg had no effect on the rate of GC. However, GW6471 suppressed anticonvulsant effect of DHA in a dose-dependent manner (Table 1).

The latency to GC occurrence in the control (no vehicle/drug-injected) group was 148.7 ± 10.1 s. No significant difference was found regarding the GC latency among the experimental groups.

3.2. GW6471 inhibits the increased seizure threshold by DHA

DHA at the doses of 0.3 and 1 mM significantly increased the threshold of clonic seizure ($p < 0.05$) compared to corresponding control group (Table 2). The increase in seizure threshold by DHA 1 mM was similar to that of DHA 0.3 mM and there was no significant

Table 1
Effect of the PPARα antagonist GW6471 and docosahexaenoic acid on the incidence of clonic seizures in mice.

Treatment	Number of mice with seizure/total mice
No vehicle/drug injection (Control)	10/10
DHA vehicle (Control)	10/10
DHA 0.3 mM	4/10**
GW6471vehicle (Control)	10/10
GW6471 (1 mg/kg)	9/10
GW6471vehicle + DHA vehicle (Control)	10/10
GW6471 (0.1 mg/kg) + DHA (0.3 mM)	5/10*
GW6471 (0.3 mg/kg) + DHA (0.3 mM)	6/10*
GW6471 (1 mg/kg) + DHA (0.3 mM)	9/10

Clonic seizures were induced by intraperitoneal injection of pentylenetetrazole 60 mg/kg. DHA and its vehicle (hydroxypropyl β-cyclodextrin 10% in artificial cerebrospinal fluid) were injected via intracerebroventricular route. GW6471 and its vehicle (dimethylsulfoxide) were injected via intraperitoneal route. *$p < 0.05$ and **$p < 0.01$, compared to corresponding control group. Data were analyzed by Fisher's exact test. DHA: docosahexaenoic acid.