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RESEARCH ARTICLES

Chronic daily administration of ethyl docosahexaenoate protects against gerbil brain ischemic damage through reduction of arachidonic acid liberation and accumulation

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Abstract

Recently, we reported that dietary ethyl docosahexaenoate (Et-DHA) intake decreases the level of membrane arachidonic acid (AA), which reduces the generation of AA metabolites in ischemic gerbil brain. As an extended study, we further investigated the influence of the chronic administration of Et-DHA on free AA levels after ischemia. In addition, Na,K-ATPase activity, cation content, cerebral edema and brain damage were also evaluated. Weanling male gerbils were orally pretreated with either Et-DHA (200 mg/kg) or vehicle, once a day for 10 weeks, and subjected to transient forebrain ischemia by bilateral common carotid occlusion for 30 min. Time-course analyses revealed that pretreatment with Et-DHA, compared with pretreatment with the vehicle, significantly decreased the brain's free AA levels during ischemia (5, 15 and 30 min) and after reperfusion (5, 10, 15 and 30 min), and attenuated the decline of Na,K-ATPase activity at examined time points. Pretreatment with Et-DHA significantly prevented an increase in Na^+ concentration and a decrease in K^+ concentration after 24 h of reperfusion, which resulted in lower cerebral water content. Reduced brain infarct volume and low animal mortality were also observed in Et-DHA-treated animals. These data suggest that the reduction of ischemia-induced AA liberation and accumulation by Et-DHA pretreatment may be attributable to (a) protection against the decline of Na,K-ATPase activity, (b) postischemic cerebral edema and brain damage and (c) animal mortality.

Keywords: Ethyl docosahexaenoate; Arachidonic acid; Na,K-ATPase activity; Cerebral edema; Brain damage; Transient forebrain ischemia

1. Introduction

Previous studies have demonstrated the effects of cerebral ischemia on free fatty acids in the brain [1,2]. It is well known that arachidonic acid (AA; 20:4*n*−6), comprising the major portion of free fatty acids, is liberated from membrane phospholipids by phospholipase after ischemia [1–3]. There is an agreement that free AA is either reesterified to phosphatidylinositol and phosphatidylcholine, as well as polyphosphoinositides, for the renewal of membrane lipids after ischemia–reperfusion [3,4], or is oxidized by lipooxygenases and cyclooxygenases, producing prostaglandins (PGs), leukotrienes and other bioactive eicosanoids [5,6]. Free AA accumulation during ischemia correlated with time for ATP depletion [7] because ATP is required for the activation of free AA to its acyl-CoA and for the reincorporation of liberated AA into phospholipids [8]. The accumulation of free AA and its metabolites induces pathological events such as membrane destruction [6,9], which may affect the activities and functions of different ion channels, transporters and other membrane-associated enzymes, particularly the most vulnerable — Na,K-ATPase, since its activity is very sensitive to even small changes in the plasma membrane lipid microenvironment [9,10]. Na,K-ATPase normally maintains intracellular and extracellular Na^+ and K^+ concentrations. The transmembrane homeostasis of these ions helps to regulate cell volume through a process described as the 'double-Donnan pump leak' hypothesis [11]. The decline of Na,K-ATPase activity after ischemia leads to cellular edema, which may ultimately contribute to cell death [12]. Therefore, the reduction of free AA and its metabolites could have a protective benefit in the treatment of cerebral ischemia.

In the absence of a selective inhibitor of AA liberation, a potential means of investigating the involvement of AA in

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ischemic brain damage is the modification of dietary fatty acid composition, and localized changes in membrane fatty acid composition may be of critical importance in determining neuronal function and responses to insults [13]. It has been shown that modest supplementation with $n-3$ fatty acids, such as eicosapentaenoic acid (20:5$n-3$) and docosahexaenoic acid (DHA; 22:6$n-3$), can alter the membrane fatty acid composition of the brain as these fatty acids compete with AA for esterification into cellular phospholipids [14]. Numerous studies have investigated how dietary $n-3$ fatty acids may be able to ameliorate some of the deleterious symptoms associated with ischemia [15]. Minami et al. [16] reported that dietary DHA significantly suppressed age-dependent increase in systolic blood pressure and prolonged the average survival time of stroke-prone spontaneously hypertensive rats. The effects were accompanied by an increase of DHA levels and a decrease of AA levels in the plasma and brain cortex. Furthermore, Okada et al. [17] showed that chronic administration of DHA contributes to protection against neuronal damage in the rat hippocampal CA1 region and reduces cognitive deficit caused by transient forebrain ischemia. They suggested that the decrease in AA content in the brain may be attributed to the protective effect of DHA treatment on neuronal damage. Our recent study demonstrates that chronic administration of ethyl docosahexaenoate (Et-DHA), the form of esterified DHA that is more effectively absorbed and incorporated into tissues than its free form [18], reduces the generation of the eicosanoids of AA metabolism following ischemia and reperfusion in gerbil brain [19]. However, the effects of Et-DHA pretreatment on the ischemia-induced changes of free AA levels, which are correlated with the pathogenesis of cerebral ischemia, were not clarified.

The aim of the present study, therefore, was to investigate the effect of the chronic daily administration of Et-DHA on free AA levels in gerbil brain at different time points following 30 min of transient cerebral ischemia. In addition, the effects of Et-DHA on Na,K-ATPase activity, Na^+ and K^+ concentrations, cerebral water content, brain infarct volume and animal mortality were also examined, allowing us to probe in vivo whether or not Et-DHA pretreatment for protective action against cerebral ischemia could be associated with the reduction of free AA liberation and accumulation.

2. Materials and methods

2.1. Animals and treatments

Weanling male Mongolian gerbils (21 days old; the Experimental Animal Center of the Zhejiang Medical University, China) were randomly divided into two groups. One group (Et-DHA group) was orally treated with Et-DHA (98% pure; Harima Chemicals, Tokyo, Japan) emulsified in 5% gum Arabic solution at a dose of 200 mg/kg (1 ml/kg), once a day for 10 weeks [19]; the other group (vehicle group) was treated with a similar volume of vehicle alone. Before and after ischemia or reperfusion, gerbils were housed six per cage at a constant room temperature of 21–22°C under a light/dark cycle of 12/12 h (0700 h/1900 h). The animals were allowed free access to food pellets and drinking water. Adaptation and experiments were carried out in accordance with internationally accepted principles and with national laws concerning the care and use of laboratory animals, and were approved by the Ethical Committee of the University of Nanjing.

2.2. Surgical preparation

At the termination of treatment, the gerbils (40–60 g) were anesthetized by inhalation of 2% halothane in 30% oxygen/70% nitrous oxide. A midline ventral incision was made on the neck, and both common carotid arteries were exposed, separated carefully from the vagus nerve and occluded with atraumatic aneurysm clips for 30 min. The same surgically operated animals without carotid occlusion served as sham animals. The duration of anesthesia administration was less than 5 min. Recirculation was visually confirmed after removal of the clips. Rectal and brain temperatures (measured by a rectal probe and a tympanic probe, respectively) were maintained at 37.0±1.0°C during occlusion and during the early period of recirculation by placing the animal in a heated box with a controlled heating lamp [20].

In studies during occlusion, the animals of both vehicle-treated and Et-DHA-treated groups were decapitated at 0, 5, 15 or 30 min from the onset of ischemia, while the clips were still in place. In recirculation studies, the animals of both groups were decapitated at 5, 10, 15, 30 or 60 min of reflow. At the predetermined time of sacrifice, the animals were briefly reanesthetized with halothane, and the scalp over the cranial vault was excised to facilitate the freezing of the brain [21]. Immediately following decapitation, the heads were immersed in liquid nitrogen with vigorous agitation. Six gerbils were assigned at each time point to both vehicle-treated and Et-DHA-treated groups. Animals dying prior to the predetermined time of sacrifice were discarded. The cerebral hemispheres were removed under intermittent application of liquid nitrogen. Cerebral tissues were stored at −80°C until biochemical assay.

2.3. Extraction and analysis of free AA

Frozen tissues were weighed and homogenized in a chloroform/methanol (2:1, vol/vol) mixture for lipid extraction [22]. Nonadecanoic acid (19:0; Sigma) was added as an internal standard. An aliquot of lipid extract was separated by one-dimensional thin-layer chromatography (TLC) on silica gel-60 (E. Merck, Germany). Petroleum ether/ether/acetic acid (97:3:1, vol/vol/vol) and petroleum ether/ether/acetic acid (80:20:1, vol/vol/vol) were used as developing solvents for free fatty acids [6]. Bands were visualized with 1% 2′ 7′ -dichlorofluorescein in methanol solution under a UV lamp and were identified by comparison with fatty acid

standards. Free fatty acid bands were removed from the plate and converted to fatty acid methyl esters (FAMEs) by acid methanolysis with BF_3-methanol (Sigma) at 60°C for 1 h [15]. FAMEs were analyzed with 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 rise from 170°C to 240°C, and detector temperature was set at 270°C. Identification of FAMEs was made by the 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. Free AA level was quantified using an internal standard and was expressed as micromoles per kilogram of wet weight.

2.4. Determination of Na,K-ATPase activity

Na, K-ATPase activity in cerebral hemisphere was measured according to the method of Post and Sen [23]. Tissue homogenates for Na,K-ATPase assays were prepared in 5–10 vol of 1 mM Tris buffer (pH 7.3) with 0.32 M sucrose (4°C) using a motor-driven (Tri-R Instruments) Teflon pestle and a glass homogenizer tube. The standard reaction mixture contained 5 mM EDTA, 20 mM imidazole-glycyl glycine buffer (pH 7.7), 3 mM ATP (Sigma), a homogenate containing 0.2–0.5 mg of protein, 100 mM Na and 20 mM K (with or without 0.17 mM ouabain). Incubations were performed in triplicate and carried out for 10 min at 37°C following a 4-min preincubation at 37°C. P_i was measured by the method of Fiske and Subbarow [24]. Preliminary experiments established that Na,K-ATPase activity was a linear function of protein concentration under these conditions. Protein concentration in homogenates was estimated by the method of Lowry et al. [25] using bovine serum albumin as standard. Na,K-ATPase activity was defined as the difference in the amount of P_i liberated in the presence and in the absence of ouabain, and was expressed as micromoles of P_i per hour per milligram of protein.

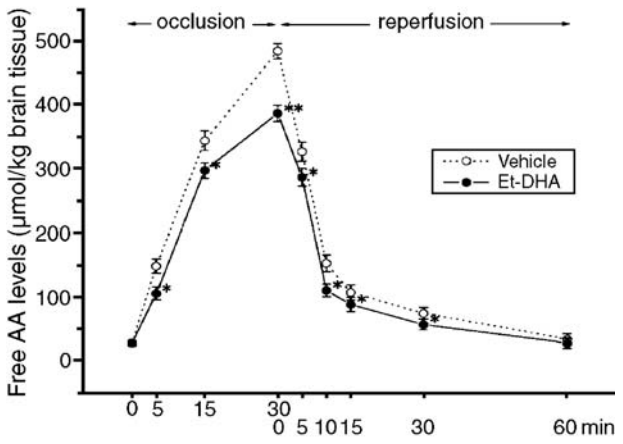

Fig. 1. The effects of pretreatment (10 weeks) with vehicle or Et-DHA (200 mg/kg/day, po) on free AA levels in gerbil brains subjected to 30 min of bilateral carotid occlusion followed up to 60 min of recirculation. Each value represents the mean±S.E.M. of six animals. *P<.05; **P<.01 versus vehicle. Statistical analysis was performed with Student' t test.

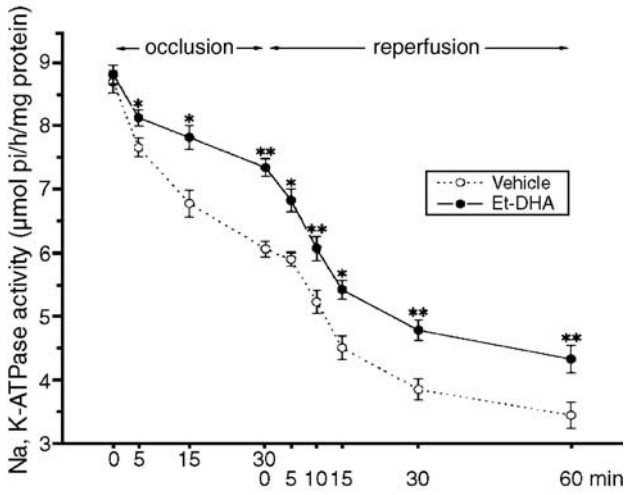

Fig. 2. The effects of pretreatment (10 weeks) with vehicle or Et-DHA (200 mg/kg/day, po) on brain Na,K-ATPase activity in gerbils subjected to 30 min of bilateral carotid occlusion followed up to 60 min of recirculation. Each value represents the mean±S.E.M. of six animals. *P<.05; **P<.01 versus vehicle. Statistical analysis was performed with Student' t test.

2.5. Evaluation of brain water, Na^+ content and K^+ content

Cerebral edema was evaluated by measuring brain water content after 24 h of reperfusion, using wet weight/dry weight ratio [26]. Freshly dissected cerebral hemispheres were weighed, dried for 24 h at 105°C and reweighed. The percentage of water (% H_2O) was calculated as 100×(wet weight−dry weight)/wet weight.

After the homogenization of dried cerebral tissues in 0.75 M HNO_3, the homogenates were maintained for 1 week at 4°C. Na^+ and K^+ contents were determined by flame photometry (IL493 Automatic Flame Photometer, USA) and were expressed as milliequivalents per kilogram of dry weight.

2.6. Measurement of brain infarct volume

For the detection of the ischemic infarct area of the brain, the cross-sectional infarct area on the surface of each brain slice was defined by 2,3,5-triphenyltetrazolium chloride (TTC) staining [27]. After 24 h of reperfusion, the gerbils received an intracardiac perfusion of 0.9% buffered saline. The brain was then removed and cut into 2-mm serial slices starting 1 mm from the frontal pole. Coronal slices were then immersed in a 2% phosphate-buffered solution for 50 min at 37°C. After TTC staining, the slices were fixed in 10% phosphate-buffered formalin. The infarct area (mm^2) from each slice was quantified with a computer-assisted image analysis system (Olympus, Tokyo, Japan), and infarct volume (mm^3) was calculated as follows: ∑slice areas (seven slices in all)×thickness (2 mm).

2.7. Detection of mortality

A mortality study using 40 gerbils for the vehicle-treated and Et-DHA-treated groups, respectively, was carried out. All

Table 1
The effects of pretreatment (10 weeks) with vehicle or Et-DHA (200 mg/kg/day, po) on Na^+, K^+ and cerebral water contents in gerbil brains at 24 h after ischemia–reperfusion

Parameters	Sham	Vehicle	E-DHA
Water content (%)	78.13±0.26	81.42±0.36*	78.35±0.57*
Na^+ content (mEq/kg dry weight)	182±18	327±24**	219±13*,***
K^+ content (mEq/kg dry weight)	501.6±15	443.5±35	487.6±26***

Values are presented as mean±S.E.M.
* $P<.05$.
** $P<.05$ versus sham.
*** $P<.01$ versus vehicle (one-way ANOVA followed by Fisher's protected LSD post hoc test).

animals underwent 30 min of bilateral carotid occlusion. Surviving animals were then recirculated, and behavioral observations were made. The number of deaths was recorded every half hour for the first 3 h of recirculation and hourly thereafter. Mortality was estimated after 24 h of reperfusion.

2.8. Statistical analysis

Data were expressed as mean±S.E.M. Student's *t* test was used to analyze the differences between two groups. Bonferroni adjustment was used for testing at two points. When more than two groups were compared, the significance of the difference between groups was evaluated by one-way analysis of variance (ANOVA) with Fisher's protected LSD post hoc test for multiple comparisons. $P<.05$ was considered significant. Fisher's exact probability test was used to compare mortality between the groups.

3. Results

3.1. Free AA levels

As shown in Fig. 1, the free AA levels of the brain increased rapidly during the course of bilateral carotid occlusion. A comparison of the levels in vehicle-treated versus Et-DHA-treated animals revealed that the increased free AA was suppressed significantly in Et-DHA-treated animals at 5 min ($P<.05$), 15 min ($P<.05$) or 30 min ($P<.01$) of occlusion. Free AA levels began to decrease after recirculation and returned nearly to their preischemic levels at 60 min of reperfusion. When compared to vehicle-treated animals, Et-DHA-treated animals had lower free AA levels at 5 min ($P<.05$), 10 min ($P<.05$), 15 min ($P<.05$) or 30 min ($P<.05$) of reperfusion, except for the difference of free AA levels at 60 min of reperfusion, which did not reach statistical significance ($P>.05$). No significant difference ($P>.05$) in free AA levels in the brain was found between the vehicle-treated group and the Et-DHA-treated group prior to carotid occlusion.

3.2. Na, K-ATPase activity

A significant reduction of Na,K-ATPase activity manifested in the course of occlusion and recirculation (Fig. 2). Et-DHA significantly prevented the activity of Na,K-ATPase from declining at 5 min ($P<.05$), 15 min ($P<.05$) or 30 min ($P<.01$) of occlusion, as compared with the vehicle. During recirculation, Et-DHA-treated animals still had higher Na,K-ATPase activities at 5 min ($P<.05$), 10 min ($P<.01$), 15 min ($P<.05$), 30 min ($P<.01$) or 60 min ($P<.01$) of reperfusion than vehicle-treated animals. However, the activities of Na,K-ATPase in Et-DHA-treated animals prior to bilateral carotid occlusion did not differ significantly from those of vehicle-treated animals ($P>.05$).

3.3. Na^+ and K^+ contents

Na^+ content in Et-DHA-treated animals was significantly lower ($P<.01$) — but K^+ content was significantly higher ($P<.01$) — than that in vehicle-treated animals. Moreover, the net ion shift caused by the difference in Na^+ influx (from 182±18 to 219±13 mEq/kg dry weight) and K^+ efflux (from 501.6±15 to 487.6±26 mEq/kg dry weight) in the Et-DHA group was much lower than that in the vehicle group (Na^+ influx: from 182±18 to 327±24 mEq/kg dry weight; K^+ efflux: from 501.6±15 to 443.5±35 mEq/kg dry weight).

3.4. Cerebral edema

The brain water content (an index of cerebral edema) observed after 24 h of reperfusion was significantly lower

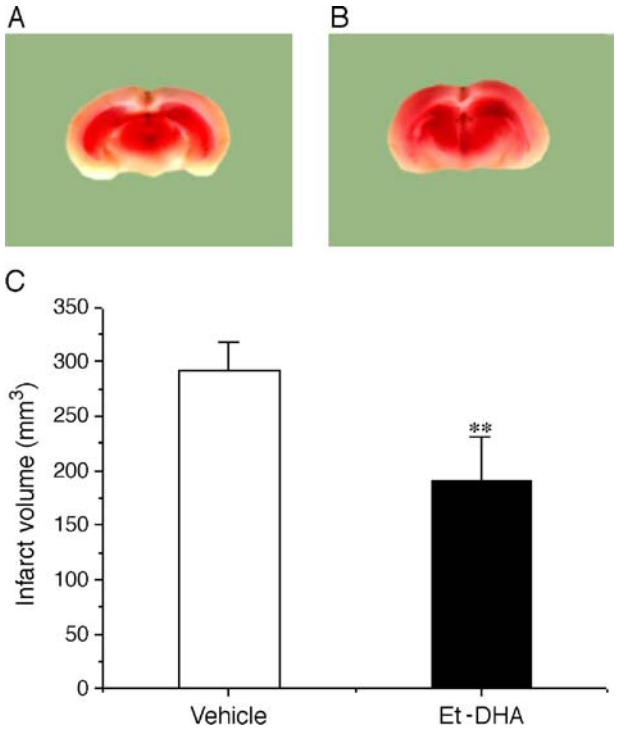

Fig. 3. The effects of pretreatment (10 weeks) with vehicle or Et-DHA (200 mg/kg/day, po) on brain infarct in gerbils at 24 h after ischemia–reperfusion. The brain infarct in brain slices cut 6 mm away from the frontal pole was detected by TTC staining. (A) Vehicle group. (B) Et-DHA group. (C) Infarct volume (mm^3). (A) and (B) show significant infarcts mainly in brain cortex sections. Each column represents the mean±S.E.M. of six animals. **$P<.01$ versus vehicle. Statistical analysis was performed with Student' *t* test.

Table 2
The effects of pretreatment (10 weeks) with vehicle or Et-DHA (200 mg/kg/day, po) on mortality in gerbils at 24 h after ischemia–reperfusion

Treatment	Mortality [n (%)]
Vehicle (n=40)	30 (75)
Et-DHA (n=40)	18 (45*)

* P<.05 versus vehicle (Fisher's exact probability test).

(P<.05) in the Et-DHA group than in the vehicle group (Table 1). Et-DHA pretreatment produced a significant reduction of postischemic cerebral edema. No significant difference (P>.05) in brain water content was found between the Et-DHA group and the sham group.

3.5. Brain infarct volume

Thirty minutes of ischemia and reperfusion resulted in clearly delineated infarcts in the cortex (Fig. 3A and B). Et-DHA-treated animals sustained smaller brain infarcts after 24 h of reperfusion (Fig. 3C), such that the total volume of damage was significantly reduced by 35% (P<.01) in Et-DHA-treated gerbils (189.1±41.4 mm^3) compared to those treated with vehicle (292.3±24.8 mm^3).

3.6. Animal mortality

Results presented in Table 2 showed that the difference in mortality between Et-DHA-treated and vehicle-treated gerbils was significant (18 of 40 versus 30 of 40, respectively; P=.012, Fisher's exact probability test) after 24 h of reperfusion. The mortality observed in Et-DHA-treated animals was significantly reduced by 40% (P<.05) compared to that seen in vehicle-treated animals. Deaths at 24 h in both groups were commonly preceded by clinical convulsions.

4. Discussion

The results of the present study showed that chronic administration of Et-DHA significantly reduced the levels of free AA during cerebral ischemia and reperfusion, which produced beneficial effects on ischemic gerbils. AA is liberated from phospholipids mainly by the action of phospholipase A_2 (PLA_2) [2,3]. Under physiological conditions, PLA_2 liberates AA from membrane phospholipids at a rate less than or equal to the rate of free AA reincorporation into membranes. Thus, a balance between the liberation of AA from, and its reacylation into, membrane phospholipids results in low levels of free AA [3,28]. However, under conditions of ischemia, free AA production exceeds utilization, and accumulation ensues [29]. A rapid increase in free AA during ischemia is generally considered as due to impaired phospholipid reacylation secondary to energy failure/ATP depletion because the reacylation process is an energy-requiring process [30,31]. Simultaneously, ischemia-induced glutamate release contributes to increased free AA because glutamate receptor stimulation leads to phosphoinositide hydrolysis and PLA_2 activation, which results in further phospholipid degradation and free AA liberation and accumulation [30,32]. The liberated and accumulated AA is not entirely recycled back into phospholipids; a certain amount is lost to eicosanoids, â-oxidation or other metabolites [33].

Free AA or its subsequent metabolites have been shown to correlate with the severity of ischemic damage [34]. Free AA resulting in brain cellular injury may result from a variety of mechanisms, including disruption of mitochondrial integrity and function [29], contribution to cellular edema [35], inhibition of transmitter and amino acid uptake and/or ion channel activity [36,37], release of intracellular calcium from the endoplasmic reticulum [38] and/or direct actions as detergents and/or ionophores [39]. Therefore, the reduction of free AA level is often used as a marker to evaluate the therapeutic benefits of interventions in cerebral ischemia–reperfusion injury.

It is well established that $n-3$ fatty acids can alter membrane fatty acid composition of the brain. Chronic $n-3$ fatty acid deprivation does not decrease AA turnover, but does not decrease DHA and the ratio of DHA/AA turnover in rat brain phospholipids, suggesting that deesterification/reesterification cycles for AA and DHA are independent of each other. However, whether chronic $n-3$ fatty acid supplementation would increase the turnover of DHA is not known [40]. The results from Okada et al. [17] and our recent study [19] suggested that chronic administration of DHA or Et-DHA significantly decreased the content of brain AA. In the present study, we demonstrated that pretreatment with Et-DHA significantly reduced free AA levels in the brain after 30 min of cerebral ischemia. The possible explanation for the effect of Et-DHA on the decrease of free AA could be a reduction in the amount of membrane AA through Et-DHA pretreatment, which inevitably reduces the amount of free AA generated after ischemia. In addition, it was reported that the activity of PLA_2, a rate-limiting step in the release of AA from membrane phospholipids, could be reduced by DHA [41], which, too, can reduce free AA liberation and accumulation during ischemia and the availability of AA for metabolism into various eicosanoids after reperfusion.

Previous studies have reported a significant decrease in brain Na,K-ATPase activity after ischemia [6,10,42]. There are several reasons for the ischemia-induced fall in Na,K-ATPase activity, such as the decline of ATP content and increased production of enzymatic inhibitors [43]. Free AA is one of the more potent Na,K-ATPase inhibitors [43,44]. According to our results, the increase in free AA level during ischemia and at 30 min of reperfusion could be related to an early decline in Na,K-ATPase activity. The protection of Et-DHA pretreatment against Na,K-ATPase activity could be due to the reduction of free AA levels in that period. Additionally, PGs are also potent inhibitors [43]. $PGF_{2\alpha}$ has been reported to be an extremely potent inhibitor of Na,K-ATPase [43,45]. DHA appears to inhibit PG biosynthesis [46]. We have recently demonstrated that

chronic administration of Et-DHA significantly reduced the levels of $PGF_{2\alpha}$ in ischemic gerbil brain [19], which could also give an explanation for the significant protection of Na,K-ATPase activity observed after 60 min of reperfusion in this study.

Na,K-ATPase is highly concentrated in the neuronal cell membrane and is important for cellular function. Membrane-bound Na,K-ATPase primarily executes uphill fluxes of Na^+ (out) and K^+ (in), and, secondarily, the extrusion of both Ca^{2+} and H^+ ions. Since ATPase creates a large electrochemical potential for Na^+, backleakage of Na^+ can drive the efflux of Ca^{2+} and H^+ via the operation of suitable antiporters [47]. Another functional role of Na,K-ATPase is the regulation of neuronal cell volume. According to the double-Donnan pump leak hypothesis [11], the presence of impermeable intracellular anions favors the influx of permeable ions across the neuronal membrane, with imbibation of water. This is prevented by energy-dependent extrusion of Na^+, which then effectively becomes an impermeable ion, balancing the presence of nonpermeating intracellular anions. However, since Na^+ can leak back into the cell, the actual neuronal cell volume is determined by the balance between the leak (Na^+ influx) and the pump (Na^+ extrusion). One can envisage, therefore, that cells would swell due to either increases in Na^+ permeability or reduction in Na^+ pump. In this study, Et-DHA pretreatment significantly prevented Na^+ from increasing — and K^+ from decreasing — by protecting against the decline of Na,K-ATPase activity and by causing a lower net ion shift, which resulted in less cerebral edema formation. The inhibition of PGs and other AA metabolites by Et-DHA may also be attributed to the effect of Et-DHA on attenuating cerebral edema. In addition, Serhan [48] recently suggested that endogenous DHA was converted in vivo to a 17*S* series of resolvins (RvD1–RvD6) as well as to 10,17*S*-docosatriene (DT), which may play an important role in the reduction of cerebral edema formation after cerebral ischemia. Our recent study indicated that pretreatment with ET-DHA significantly increased brain lipid DHA content [19], which may contribute to the production of 17*S*-containing DT and RvD. The contribution of this possible action of ET-DHA to the reduction of postischemic cerebral edema cannot be ruled out.

Cerebral edema causes a mass effect and aggravates primary ischemic injury in the brain [49]. The suppression of Na,K-ATPase activity is responsible for the aggravation of ischemic neuronal damage [50]. Free AA metabolites derived from both the cyclooxygenase and lipoxygenase pathways are of theoretical importance in the development of infarct after cerebral [51]. In the present study, the effects of Et-DHA on cerebral edema, and the activity of Na,K-ATPase and free AA level would give possible explanations for the reduced brain infract and the lower mortality observed in Et-DHA-treated gerbils. Furthermore, the novel DHA-derived DT mentioned above is shown to be a potent inhitbitor of ischemia–reperfusion-induced polymorphonuclear neutrophil infiltration and proinflammatory gene induction, which potently elicited neuroprotection in vivo by reducing stroke infarct volume after middle cerebral artery occlusion and reperfusion in rats [52]. Moreover, Et-DHA has been found to enhance free radical scavenging in rat fetal brain [53] or in ischemic gerbil brain [54], and DHA has been shown to have a protective effect against glutamate-induced neurotoxicity in vitro [55]. All these effects may also lead to the beneficial actions of Et-DHA in protecting against brain damage and animal death.

5. Conclusion

The chronic administration of Et-DHA significantly decreased free AA levels in the brain, which produced significant protection of Na,K-ATPase activity and subsequent benefits in reducing Na^+ and K^+ contents, cerebral edema, brain infarct and animal mortality in ischemic gerbils. Although the mechanisms responsible for protection (by Et-DHA pretreatment) against ischemic damage are unclear, the present study strongly suggests that the reduction of free AA liberation and accumulation after cerebral ischemia may be partly involved in the mechanism underlying such protection.

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