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Research Report

Docosahexaenoic acid and phosphatidylserine supplementations improve antioxidant activities and cognitive functions of the developing brain on pentylenetetrazol-induced seizure model

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ABSTRACT

Epilepsy provoked by pentylenetetrazol (PTZ) is caused by an abnormal excitatory postsynaptic potential, which results in increased production of reactive oxygen species, and finally reducing cognitive functions. The objective of this study was to investigate the effects of dietary supplementation with DHA and PS, administered either alone or in combination, on oxidative stress and behavioral and cognitive spatial memory in neonatal rats with PTZ-induced epileptic seizure. In this study, rat pups received repetitive doses of PTZ for induction of epileptic seizure and docosahexaenoic acid (DHA, C22:6, n−3) and phosphatidylserine (PS) were orally administrated alone or together to the PTZ-induced epileptic animals daily for 36 d. The spatial memory, nitric mono-oxide (NO) production, and enzymatic activities of superoxide dismutase (SOD) and catalase in brain and liver tissues were determined. PTZ administration significantly reduced the cell numbers in the hippocampus, shortened the escape latency in the safe target region, decreased activities of SOD and catalase, but increased NO content in both brain and liver tissues, while DHA and PS significantly extended the escape latency, reversed the oxidative parameters observed in the brain, and enhanced SOD activity in the liver. Dietary supplementation with DHA and PS may protect brain tissue from the oxidative stress caused by epileptic seizures and could serve to improve learning and memory ability *in vivo*.

1. Introduction

Seizures are self-sustained, usually time-limited, episodes of neuronal hyperexcitability, involving synchronous discharges in large neuronal populations (Burnham, 2007). It has been observed that the neuropathologic cellular changes during sustained epileptic activity in the hippocampus are characterized by an intense transient influx of calcium leading to mitochon-

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drial functional impairments (Heinemann et al., 2002; Patel, 2002) and also by the triggering of intracellular enzyme cascades, including the activation of nitric oxide synthase, which leads to increased levels of reactive oxygen species (ROS) and oxidative stress (Farooqui et al., 2001; Lafon-Cazal et al., 1993). Formation of free radicals results in extensive lipid peroxidation, which damages cellular organelles and membranes, and finally leads to cell death. Thus, oxidative stress has been well known to be a significant consequence of excitotoxicity, which plays a critical role in epileptic brain damage.

It has been reported that chronic administration of n−3 polyunsaturated fatty acids (PUFA) may increase resistance to pentylenetetrazol (PTZ)-induced seizures in rats (Yehuda et al., 1994), possibly by raising brain levels of the docosahexaenoic acid (DHA; C22 : 6n−3) (Yehuda et al., 1996). DHA, the final compound in the n−3 PUFA synthetic pathway and the most abundant n−3 PUFA found in the brain, is essential for brain growth (Ratzliff et al., 2002) and plays important roles in increasing membrane fluidity, improving neurogenesis and synaptogenesis (Guo et al., 2007), and scavenging the intracellular radical productions induced by H_2O_2 radicals $O_2.^-$, and .OH (Shimazawa et al., 2009).

A previous study has shown that DHA positively modulates phosphatidylserine (PS) biosynthesis and accumulation in neuronal cells promoting survival, and that it inhibits apoptosis in a PS-dependent manner (Ratzliff et al., 2002). PS plays an important role in modulating neuronal membrane function, and it is also important for the maintenance of the cell's internal environment, signal transduction patterns, secretory vesicle release, intra-cellular communication, and growth regulation (Gianotti et al., 1993; Nishizuka, 1984; Pedata et al., 1985; Toffano et al., 1978; Vannucchi and Pepeu, 1987). Treatment with PS enhances cholinergic neurotransmission, restores acetylcholine release in aging rats (Casamenti et al., 1991; Chung et al., 1995; Nishizuka, 1984), increases the turnover of dopamine and norepinephrine in the brain (Toffano et al., 1978), and also enhances Na^+, K^+, and ATPase activity in the brain (Wheeler and Whittam, 1970). Clinical studies have provided evidence that PS could possibly improve cognitive function in demented and aged patients (Crook et al., 1992; Delwaide et al., 1986). Cenacchi et al. (1993) investigated 494 elderly patients and demonstrated the potential benefit of taking PS as an anti-dementia agent, as improved behavior and cognitive performance was observed in these patients without side effects. Animal studies also demonstrated the cognition-enhancing properties of sub-chronic PS treatment in middle-aged rats (Blokland et al., 1999). Although there are many studies focused on PS and its effects on the aged brain, only very few studies have investigated PS effects on the developing brain. The aim of this study is to investigate the effects of dietary supplementation with DHA and PS, administered either alone or in combination, on oxidative stress and behavioral and cognitive spatial memory in neonatal rats with PTZ-induced epileptic seizure.

2. Results

The experimental animals showed typical seizure behaviors, including convulsive waves of the limbs and turning over onto the back position, after receiving PTZ as described in the PTZ-induced seizure animal model (Zhang et al., 2003). Significantly reduced cell numbers in CA1 and CA3 of the hippocampus (Fig. 1), diminished escape latency in Morris water maze test, along with decreased SOD activity in brain and liver tissues, and decreased catalase activity in liver tissues were observed in rats with the PTZ-induced seizures compared with those same parameters observed in the normal controls (Fig. 2). Moreover, NO levels in both brain and liver tissues of rats with PTZ-induced seizures were significantly higher than normal controls (Fig. 2).

Dietary supplementation with DHA or PS alone significantly increased the escape latency time of the seizure-induced animals as compared with that of the saline controls ($P<0.05$); however, significantly synergistic effects of combined DHA/PS supplementation was not observed (Fig. 3).

DHA and PS administered individually also significantly enhanced SOD activities in brain tissue; again, no additive effects of combined DHA/PS administration were observed (Fig. 4A). However, the elevated liver SOD activity was observed only in PTZ-treated animals with both DHA and PS supplementation, and not in those supplemented with DHA or PS alone (Fig. 4B).

In the PTZ-induced epileptic animals, dietary supplementation with DHA, PS or DHA/PS together significantly enhanced catalase activities in both the brain and liver tissues as compared with activities of these enzymes observed in the saline controls (Fig. 5).

Combined DHA/PS supplementation significantly reduced NO levels in the brain tissues of PTZ-treated animals rather than supplementation with either DHA or PS alone, as compared with levels of the saline controls (Fig. 6A). In the livers of the PTZ-treated animals, supplementation with DHA, PS and DHA/PS significantly decreased the NO production (Fig. 6B).

3. Discussion

Epilepsy has been described as a condition of excessive neuronal discharge associated with or resulting from oxidative stress (Shin et al., 2011; Waldbaum and Patel, 2010). Although the causality between epilepsy and oxidative stress has not yet been fully clarified, epileptic biomarkers, such as behavior traits, catalytical antioxidants, and oxidative products in neuronal tissues have been monitored for evaluation of the degree of epilepsy and epileptic pathogenesis. PTZ treatment is commonly used as an animal model for induction of the status of epilepsy (Loscher, 2011; Nehlig and Pereira de Vasconcelos, 1996), and PTZ administration may effectively introduce oxidative stress to the neuronal and liver tissues (Akbas et al., 2005; Dillioglugil et al., 2010). In the present study, the escape latency percentage of animals with PTZ-induced seizures was only one-third that of the normal controls, while their NO content in brain tissue was significantly enhanced nearly threefold. In addition, the SOD activity in the PTZ-treated animals was reduced to one-eighth and one-third the control group levels in the brain and liver tissues, respectively. These results are consistent with the observation that nearly a fivefold elevation in NO content was found at