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Research Report

Therapeutic impact of eicosapentaenoic acid on ischemic brain damage following transient focal cerebral ischemia in rats

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ABSTRACT

Long-chain n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA), have been shown to reduce ischemic neuronal injury. We investigated the effects of ethyl-EPA (EPA-E) on ischemic brain damage using a rat transient focal cerebral ischemia model. Male Sprague–Dawley rats ($n=105$) were subjected to 90 min of focal cerebral ischemia. EPA-E (100 mg/kg/day) or vehicle was administered once a day for 3, 5 or 7 days prior to ischemia. Different withdrawal intervals of 3, 5, and 7 days prior to ischemia following 7-day pretreatment with EPA-E or vehicle were also examined. In addition, post-ischemic administration of EPA-E was investigated. Pretreatment with EPA-E for 7 and 5 days, but not 3 days, showed significant infarct volume reduction and neurological improvements when compared with vehicle pretreatment. In addition, withdrawal of EPA-E administration for 3 days, but not 5 and 7 days, also demonstrated significant infarct volume reduction and neurological improvements when compared with vehicle treatment. Post-ischemic treatment of EPA-E did not show any neuroprotection. Immunohistochemistry revealed that 7-day pretreatment with EPA-E significantly reduced cortical expression of 8-hydroxydeoxyguanosine (maker for oxidative DNA damage), 4-hydroxy-2-nonenal (maker for lipid peroxidation), phosphorylated adducin (marker for Rho-kinase activation) and von Willebrand factor (endothelial marker) when compared with vehicle pretreatment. In addition, phosphorylated adducin expression co-localized with von Willebrand factor immunoreactivity. The present study established the neuroprotective effect of EPA-E on ischemic brain damage following transient focal cerebral ischemia in rats, which may be involved in the suppression of oxidative stress and endothelial Rho-kinase activation.

1. Introduction

Long-chain n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are derived from marine products, have attracted considerable attention. Early epidemiological studies in Greenland discovered that the Inuit people showed a lower incidence of myocardial infarction than Danish people (Bang et al., 1971),

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and EPA from a fish-rich diet was considered to be responsible for preventing thrombosis and atherosclerosis in the Inuit (Dyerberg et al., 1978). Furthermore, a recent meta-analysis of cohort studies disclosed an inverse relationship between fish consumption and stroke risk (He et al., 2004). A large amount of highly purified EPA can be prepared in an ethyl-ester form (EPA-E) with an approximately 90% purity (Mizuguchi et al., 1993), and EPA-E has been clinically used as a lipid-lowering medication in Japan. In addition, a recent clinical controlled trial of the Japan EPA lipid intervention study (JELIS) has shown that EPA-E reduces stroke recurrence in Japanese hypercholesterolemic patients (Tanaka et al., 2008).

We have previously shown that long-term administration of EPA-E (100 mg/kg/day) ameliorated the age-related decline of cerebral blood flow (CBF) in stroke-prone spontaneously hypertensive rats (Katayama et al., 1997). Furthermore, we have also reported that post-ischemic delayed administration of EPA-E (100 mg/kg/day) for 4 weeks increased local CBF within the peri-infarct areas in a rat chronic cerebral infarction model (Katsumata et al., 1999). In addition, several investigations have described the neuroprotective effects of EPA on forebrain ischemia (Okabe et al., 2011; Bas et al., 2007; Ozen et al., 2008; Ajami et al., 2011). However, the protective effects of EPA on acute focal cerebral ischemia remain unclear.

Cerebral ischemia consists of complex pathological processes, and various factors can exacerbate ischemic brain damage. Oxidative stress is known to be involved in ischemia-reperfusion injury owing to an increase in reactive oxygen species (ROS), which result in DNA damage and lipid peroxidation (Floyd and Carney, 1992; Halliwell, 1992). In addition, Rho-kinase activity is associated with various cerebral vascular diseases, including ischemic brain injury (Chrissobolis and Sobey, 2006). A recent investigation revealed that endothelial Rho-kinase activation following cerebral ischemia played an important role for infarct expansion (Yagita et al., 2007). Furthermore, ROS-induced brain endothelial dysfunction involves the Rho-kinase signaling pathway (Kahles et al., 2007). Therefore oxidative stress and subsequent endothelial Rho-kinase activation may be implicated in ischemic brain injury.

The present study aimed to examine the effects of pre-ischemic and post-ischemic treatments of EPA-E on ischemic brain damage using a rat transient focal cerebral ischemia model. Furthermore we sought to determine whether EPA-E could suppress oxidative DNA damage, lipid peroxidation and endothelial Rho-kinase activation following focal cerebral ischemia.

2. Results

2.1. Plasma fatty acids

Plasma EPA levels were significantly elevated after 7-day pretreatment with EPA-E compared with vehicle treatment ($p=0.0019$), although plasma arachidonic acid (AA) levels were not different between the groups ($p=0.9701$) (Table 1). In experiment 2, plasma EPA concentrations were significantly higher even with withdrawal of EPA-E administration for 3 days compared with vehicle administration ($p=0.0022$); however, EPA-E withdrawal for more than 5 days showed no differences in plasma EPA levels between the groups (Table 1). Plasma AA levels were not different between the groups at any withdrawal interval. In experiment 3, plasma EPA levels were significantly elevated following high-dose EPA-E administration (600 mg/kg/day) compared with the other treatments (Table 2). In contrast, plasma AA levels were significantly decreased following high-dose EPA-E administration compared with the other treatments (Table 2). There were no statistical differences in plasma EPA and AA levels between animals treated with 100 mg/kg/day of EPA-E and vehicle (Table 2).

2.2. Magnetic resonance imaging (MRI)

Fig. 1A displays representative CBF and apparent diffusion coefficient (ADC) images during ischemia in animals subjected to focal cerebral ischemia following 7-day pretreatment with EPA-E or vehicle. Decreased CBF area in EPA-E and vehicle treated groups was $50.0\pm9.1\ mm^2$ and $48.1\pm4.0\ mm^2$, respectively. Reduced ADC area in EPA-E and vehicle treated groups was $12.7\pm7.4\ mm^2$ and $30.2\pm6.0\ mm^2$, respectively. The decreased ADC area was significantly smaller in the EPA-E treated group when compared with the vehicle-treated group ($p=0.0034$), although reduced CBF areas were not different between the groups ($p=0.6956$) (Fig. 1A).

2.3. Infarct volumes

Typical 2,3,5-triphenyltetrazolium chloride (TTC)-stained brain sections, obtained from the present study, are shown in Fig. 1B–D.

Fig. 1B displays representative TTC-stained brain sections from experiment 1. In the 3-day pretreatment group, the cortical and striatal infarct volumes (EPA-E versus vehicle) were 180.0 ± 45.8 versus $162.8\pm30.3\ mm^3$ ($p=0.5046$) and 60.8 ± 12.7 versus $53.8\pm14.1\ mm^3$ ($p=0.4339$), respectively. In the 5-day pretreatment group, the cortical and striatal infarct volumes were 51.8 ± 53.5 versus $128.9\pm41.9\ mm^3$ ($p=0.0349$) and 58.5 ± 6.2 versus $65.9\pm10.9\ mm^3$ ($p=0.2277$), respectively. In the 7-day pretreatment group, the cortical and striatal infarct volumes at 24 h after reperfusion were 80.6 ± 29.9 versus $145.6\pm33.9\ mm^3$ ($p=0.0123$) and 43.2 ± 7.2 versus $63.7\pm6.3\ mm^3$ ($p=0.0014$), respectively. In addition, the cortical and striatal infarct volumes at 72 h after reperfusion following 7-day pretreatment were 52.4 ± 47.4 versus $159.7\pm42.9\ mm^3$ ($p=0.0056$) and 36.0 ± 8.9 versus $58.7\pm4.2\ mm^3$ ($p=0.0009$), respectively. Pretreatment with EPA-E for 7 and 5 days, but not 3 days, showed significant reduction in cortical infarct volumes, compared with the vehicle pretreatment (Fig. 2). Furthermore, 7-day pretreatment with EPA-E resulted in significant infarct volume reduction even in the striatum compared with vehicle pretreatment (Fig. 2). The protective effects, observed in animals with 7-day pretreatment of EPA-E, persisted for 72 h after reperfusion (Fig. 2).

Fig. 1C shows representative TTC-stained brain sections from experiment 2. In the 3-day withdrawal group, the cortical and striatal infarct volumes (EPA-E versus vehicle) were 28.6 ± 10.9 versus $135.2\pm49.4\ mm^3$ ($p=0.0015$) and 57.7 ± 7.7 versus $62.1\pm10.1\ mm^3$ ($p=0.4683$) at 24 h after reperfusion, respectively. In the 5-day withdrawal, the cortical and striatal