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DOCOSAHEXAENOIC ACID ATTENUATES HYPERGLYCEMIA-ENHANCED HEMORRHAGIC TRANSFORMATION AFTER TRANSIENT FOCAL CEREBRAL ISCHEMIA IN RATS

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Abstract—Hemorrhagic transformation (HT) is a feared complication of cerebral ischemic infarction, especially following the use of thrombolytic therapy. In this study, we examined whether docosahexaenoic acid (DHA; 22:6n-3), an omega-3 essential fatty acid family member, can protect the brain from injury and whether DHA can decrease the risk of HT enhanced by hyperglycemia after focal ischemic injury. Male Sprague–Dawley rats were injected with 50% dextrose (6 ml/kg intraperitoneally) to induce hyperglycemia 10 min before 1.5 h of filament middle cerebral artery occlusion (MCAO) was performed. Treatment with DHA (10 mg/kg) 5 min before reperfusion reduced HT and further improved the 7-day neurological outcome. It also reduced infarct volume, which is consistent with the restricted DWI and T2WI hyperintensive area. Reduced Evans Blue extravasation and increased expression of collagen IV indicated the improved integrity of the blood–brain barrier (BBB) in DHA-treated rats. Moreover, DHA reduced the expression of the intercellular adhesion molecule-1 (ICAM-1) in the ischemic injured brain. Therefore, we conclude that DHA attenuated hyperglycemia-enhanced HT and improved neurological function by preserving the integrity of BBB and reducing inflammation. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: hemorrhagic transformation, docosahexaenoic acid, hyperglycemia.

INTRODUCTION

Hemorrhagic transformation (HT) is the major complication in intravenous thrombolytic therapy (Group, 1997; Tanahashi, 2009; Calleja et al., 2013; Jickling et al., 2014), which is considered to be the most efficacious treatment for acute ischemic stroke (Fisher and Brott, 2003; Chapman et al., 2014). The narrow therapeutic window (up to 4.5 h after symptom onset) for thrombolytic treatment and the absence of drugs affecting the coagulation status limit the use of thrombolytic therapy in some ischemic stroke patients. Moreover, recombinant tissue plasminogen activator (r-tPA) treatment increases the risk of HT especially when administered too late. Several cellular mechanisms of HT have been illustrated by previous studies, including oxidative stress (Chen et al., 2010; Llombart et al., 2014), inflammatory reaction (Rosell et al., 2008) (Liu et al., 2014) and disruption of the blood–brain barrier (BBB) due to the tight junction reduction (Scalzo et al., 2013; Jha et al., 2014; Ozkul-Wermester et al., 2014).

Docosahexaenoic acid (DHA; 22:6n-3), the precursor of neuroprotectin D1 (NPD1), is an essential omega-3-fatty acid concentrated in the central nervous system. After being systematically administrated, free DHA is transmitted through the blood stream and concentrated in synapses and cellular membranes in the brain and retina. Under oxidative stress like ischemia, DHA is converted to NPD1 through 15-lipoxygenase-1 in the nervous system and retinal pigment epithelial (RPE) cells and released from the cell membranes (Calandria et al., 2009). Rats treated with low (3.5 or 7 mg/kg) and medium (16 or 35 mg/kg) doses of DHA after middle cerebral artery occlusion (MCAO) onset showed significantly reduced infarct volumes (Belayev et al., 2009). Similar neuroprotective effect of DHA complex to albumin (DHA-Alb) was observed in permanent MCAO (Eady et al., 2012). Moreover, Hong et al. (2015) showed that DHA protected ischemia-induced BBB disruption. Since BBB disruption is closely related to HT, we hypothesize that DHA may decrease the risk of HT transformation after stroke. As hyperglycemia, BBB leakage and inflammation are closely related with HT transformation, we therefore assessed the effects of DHA on the integrity of BBB, hyperglycemia-enhanced HT and the associated

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Abbreviations: BBB, blood–brain barrier; DHA, docosahexaenoic acid; DWI, diffusion-weighted imaging; HI, hemorrhagic infarction; HT, hemorrhagic transformation; ICAM-1, intercellular adhesion molecule-1; MCAO, middle cerebral artery occlusion; NPD1, neuroprotectin D1; PBS, phosphate-buffered solution; PH, parenchymal hemorrhage; RCBF, regional cerebral blood flow; T2WI, T2-weighed images; TPA, tissue plasminogen activator; TTC, 2,3,5-Triphenyltetrazolium Chloride.

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inflammatory response after transient focal ischemia in rats.

EXPERIMENTAL PROCEDURES

Surgical preparation and procedure

All experiments were approved by the Institutional Animal Care and Use Committee of the Zhejiang University (ZJU201312-1-02-092). Male Sprague–Dawley rats weighing 280–350 g were fasted overnight but allowed free access to water before the filament MCAO was performed. The rats were anesthetized using 4% chloral hydrate (400 mg/kg, i.p). Laser-Doppler flowmetry (Periflux system 5000; Perimed, Stockholm, Sweden) was used to monitor regional cerebral blood flow (rCBF) before, during and after the surgery with the probe positioned 2 mm posterior and 5 mm lateral from the Bregma. CBF values were recorded every 10 min. The filament MCAO procedure was performed as previously described (Bederson et al., 1986). Briefly, a 3-0 nylon suture (Sunbio Biotech Ltd., Beijing) was inserted into the right common carotid artery through the internal carotid artery to cause an occlusion at the MCA. At 1.5 h after occlusion, the filament was carefully removed. Animals that did not show a significant reduction in rCBF during MCAO or rapid restoration of the laser-Doppler signal during reperfusion were excluded from the experiment. All rats were intraperitoneally injected with 50% dextrose (6 ml/kg) 5 min before MCAO to induce acute hyperglycemia according to Lou et al. (2007). The right femoral artery was catheterized to obtain blood samples for blood gas analysis before and after surgery. However, blood was obtained from the tail vein for glucose-level analysis before and after the administration of dextrose. Rectal temperature was maintained between 36 °C and 37 °C during the surgical procedures.

DHA administration

Previous studies suggest that large doses of DHA (70 mg/kg) had no protective effects, while low (3.5 or 7 mg/kg) and medium (16 or 35 mg/kg) doses decreased infarction (Belayev et al., 2009). In our preliminary studies, we tested multiple dosages of DHA (5 mg/kg, 10 mg/kg and 20 mg/kg) based on data from other reports, and found that 10 mg/kg was more effective against HT than 5 mg/kg, while the effect of the 20 mg/kg dose was similar to 10 mg/kg (data not shown). Therefore, the dosage of 10 mg/kg was chosen for the formal experiment. DHA (10 mg/kg) was dissolved in saline and administered intravenously at a constant rate over 3 min using an infusion pump 5 min before reperfusion according to the methods described by Belayev et al. (2009). Control rats received an intravenous infusion of a comparable volume of 0.1 mol/L phosphate-buffered solution (PBS). Four experiments were performed and rats were randomly assigned to the DHA-treated group and control group. In the first experiment (n = 15 per group), rats were sacrificed 24 h after MCAO and brain slices were used for HT evaluation, TTC staining and spectrophotometric assay of intracerebral hemorrhage in order. In the second experiment (n = 10 per group), rats were used for BBB permeability measurements. In the third experiment (n = 5 per group), arterial gases were measured perioperatively and MR scans were performed 24 h after reperfusion followed by immunohistochemistry. In the fourth experiment (n = 10 per group), neurological scores were evaluated at 24 h, 3 days and 7 days after MCAO.

Neurological scores

The neurological scores were evaluated by an observer blinded to the treatment groups at 24 h, and 3 and 7 days after MCAO using the Garcia scoring system reported with modifications (Garcia et al., 1995). The neurologic evaluation described by Garcia and colleagues (1995) consists of the following six tests: 1, spontaneous activity; 2, symmetry in the movement of four limbs; 3, forepaw outstretching; 4, climbing; 5, body proprioception; and 6, response to vibrissae touch. Severe impairments in each of the tests were graded 0 or 1, and no observable deficits were graded 3. The minimum neurologic score is 3, and the maximum is 18 (i.e., in intact rats).

2,3,5-Triphenyltetrazolium Chloride (TTC) staining and evaluation of infarct volume

Rats were anesthetized with 4% chloral hydrate, perfused transcardially with 0.1 mol/L PBS and sacrificed 24 h post-stroke. The brains were rapidly removed and sliced into 2-mm-thick coronal sections. We first took pictures of the whole and sliced brain to observe if HT was present. Then brain slices were immersed in 2% TTC (Sigma) for 30 min at 37 °C in the dark (Yin et al., 2003). The infarction and hemisphere area of each section were traced and measured using an Image J analysis system. The possible interference of brain edema with infarct volume was corrected by standard methods (whole contralateral hemisphere volume-non ischemic ipsilateral hemisphere volume) and the infract volume was expressed as a percentage of the whole contralateral hemisphere (Gao et al., 2006).

MR scans

MR T2-weighed images (T2WI) and diffusion-weighted imaging (DWI) were chosen to assess infarct volume and reperfusion injury. Rats were re-anesthetized 24 h after reperfusion and coronal scans were obtained using a GE Signa HD 3.0 T scanner (TR 2000 ms, TE 90 ms, field of view read 50 mm, thick 1.5 mm, 8 slices, scan time 5 min for T2WI; thick 2 mm, interval 0 mm, field of view 240 * 240 m^2, matrix 256 * 256, b value 1000, scan time 32 s for DWI).

Evaluation of HT

The subtype of HT in the coronal slice was classified according to the criteria described by Aronowski et al. (2003): hemorrhagic infarction 1 (HI-1): with scattered, heterogeneous petechiae along the margins of the infarction; hemorrhagic infarction 2 (HI-2): that is more confluent, but heterogeneous petechiae are still present within