EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Enriched Brain Omega-3 Polyunsaturated Fatty Acids Confer Neuroprotection against Microinfarction

Chuanming Luo [a,b,1], Huixia Ren [a,1], Xiaoli Yao [c], Zhe Shi [a], Fengyin Liang [c], Jing X. Kang [d], Jian-bo Wan [a], Zhong Pei [c], Kuan-Pin Su [e], Huanxing Su [a,*]

[a] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China
[b] Department of Neurology, Seventh Affiliated Hospital, Sun Yat-Sen University, Shenzhen 510080, China
[c] Department of Neurology, National Key Clinical Department and Key Discipline of Neurology, First Affiliated Hospital Sun Yat-Sen University, Guangzhou 510080, China
[d] Laboratory for Lipid Medicine and Technology, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[e] Department of Psychiatry & Mind-Body Interface Laboratory (MBI-Lab), China Medical University Hospital, College of Medicine, China Medical University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 17 February 2018
Received in revised form 10 May 2018
Accepted 23 May 2018
Available online xxxx

Keywords:
Fish oil
Cognitive decline
Vascular dementia
Neuropsychiatric disorders

ABSTRACT

Cerebral microinfarcts have significant effects on the development of geriatric neurological disorders, including vascular dementia and Alzheimer's disease. However, little is known about the pathophysiological mechanisms involved in the evolution of microinfarcts and potential treatment and prevention against these microvascular ischemic lesions. In the present study, the "single cortical microinfarct model" generated via occluding a penetrating arteriole by femtosecond laser ablation and the "multiple diffuse microinfarcts model" induced by unilateral injection of cholesterol crystals through the internal carotid artery were established to investigate the pathophysiological mechanisms underlying the evolution of microinfarcts and the effects of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) on alleviating microinfarct burdens and functional deficits. The occlusion of a single penetrating arteriole led to a distinct cortical microinfarct, which manifested as neuronal loss and occupation of activated glial cells in the ischemic core. Using *Fat-1* transgenic mice and fish oil supplements, we demonstrated that both endogenously-generated and exogenously-delivered ω-3 PUFAs significantly inhibited the activation of receptor-interacting serine/threonine protein kinases 1 (RIPK1) and its downstream apoptosis-associated proteins, mitigated cell apoptosis, and anatomically reduced the microinfarct size. The protective effects of ω-3 PUFAs against microinfarcts were further verified in a multiple diffuse microinfarcts model, where ω-3 PUFAs significantly attenuated cell apoptosis as revealed by TUNEL staining, alleviated the diffuse microinfarct burdens and remarkably improved the functional deficits as evidenced by reduced spontaneous anxiety, increased preference for the novel object, and improved hippocampal-based learning and short-term memory. Together, these findings demonstrate that enriched brain ω-3 PUFAs are effective for reducing microinfarct burdens and improving the function deficits, which support the clinical research and application of ω-3 PUFAs in the treatment or prophylaxis in vascular dementia.

1. Introduction

Accumulating evidence suggests that cerebral microinfarcts have significant effects on the development of neuropsychiatric disorders [3,29]; Knopman, 2012; [10]. In post-mortem studies, cerebral microinfarcts are abundantly detected in the brain of patients with mild cognitive impairment (MCI) [14], vascular dementia (VaD) [9], Alzheimer's disease (AD) [33], and depression [1,45]. Furthermore, advanced neuroimaging technology provides compelling anatomical evidence that cerebral microinfarcts are prevalent in aging people with various brain disorders, including VaD [27,32], MCI [15,44], depression [36], and AD [28,42].

Cerebral microinfarct is defined as microscopic vascular occlusion <1 mm (mm) in size, which results from a variety of cerebral small vessel diseases, such as vessel lumen occlusion, arteriosclerosis and vascular wall inflammation [3]. Although the definite pathogenesis of cerebral microinfarcts remains unknown, it has been proposed that neuroinflammation, oxidative stress and apoptosis might be the main triggering factors [42]. However, little is known about the pathophysiological mechanisms underlying the evolution of microinfarcts and the causal effects of microinfarcts on neuropsychiatric manifestation. With the growing health burden of dementia and depression in the aged population worldwide, it is urgently important now to understand the

* Corresponding author at: State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China.
E-mail address: huanxingsu@umac.mo (H. Su).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ebiom.2018.05.028

etiology, pathophysiological alterations and functional consequences of cerebral microinfarcts, as well as to develop effective and safe approaches for the treatment and prevention against the development of microinfarcts.

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are highly enriched in the central nervous system and serve as an important structural component to maintain cellular functional integrity [35,41]. In the past decades, a series of epidemiological studies and clinical trials have suggested that increasing dietary intake or nutritional supplementation of ω-3 PUFAs, particularly long-chain PUFAs like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is closely associated with a reduced risk to or therapeutic effects on cognitive disorders [2,12,49]. Moreover, increased intake of ω-3 PUFAs has been reported to significantly improve behavioral, neurological and histological outcomes in focal ischemic stroke models by modulating inflammatory, anti-oxidative, neurotrophic, and anti-apoptotic responses [4,6,13,30,39]. However, no previous study has investigated the effects of ω-3 PUFAs on microinfarcts. Accordingly, it is of great interests to determine whether ω-3 PUFAs can attenuate microinfarcts and improve cognitive impairments related to microinfarcts.

In the present study, we established a single microinfarct model by occlusion of a cortical penetrating arteriole with focused femtosecond laser pulses. In addition, the multiple diffuse microinfarcts model was implemented using unilateral injection of cholesterol crystals through the internal carotid artery. Our aims were to investigate the molecular and cellular responses to the formation of microinfarcts and evaluate the potential therapeutic effects of ω-3 PUFAs on alleviating microinfarcts and cognitive impairment.

2. Materials and Methods

2.1. Animals and Dietary Supplementation

All animal studies were conducted following prevailing laws and institutional guidelines on the humane care and use of laboratory animals and were approved by the ethical committee of the University of Macau and Sun Yat-Sen University. In all the experiments, 8–10-week old male *Fat1* transgenic mice and C57BL/6J mice (22–24 g) were used. Mice were housed in a temperature-controlled room at 25 °C with a 12:12 h light-dark cycle and ad libitum access to food and water. Male heterozygous *Fat1* mice were obtained from Dr. Jing X. Kang, Harvard Medical School (Cambridge, MA, USA) and used to mate with female C57BL/6 mice to generate heterozygous *Fat1* mice and wild type (WT) littermates for experimental studies. The transgenic mouse carries a *Fat1* gene that converts ω-6 PUFAs into ω-3 PUFAs, leading to the abundance of ω-3 PUFAs and a high ω-3 PUFAs /ω-6 PUFAs ratio in tissues [11]. A modified diet containing 10% corn oil (TROPHIC Animal Feed High-tech Co., Ltd., China), with a fatty acid profile high in ω-6 PUFAs (mainly linoleic acid) and low in ω-3 PUFAs (~ 0.1% of the total fat supplied) was given. Accordingly, *Fat1* mice provided a suitable model to investigate the effects of endogenous ω-3 PUFAs.

The advance proper fish oil (containing 60% DHA and 15% EPA, Wuhan Sheng Tian Yu Biotechnology CO., LTD, China) was given to adult C57BL/6 mice (30 mg/kg weight) through daily oral gavage for 3 weeks prior to the subsequent experiments. Control animals received intragastric administration of equivoluminal isocaloric corn oil daily for 3 weeks prior to the subsequent experiments.

2.2. Fatty Acid Analysis

To evaluate the effects of the expression of the *Fat1* gene and the dietary regime on the PUFA composition in the brain cortex, the cortical tissue samples of mice from the experimental groups (n = 3 per group) were dissected and processed for fatty acid analysis by gas chromatography–mass spectrometry (GC–MS). Quantifications were performed by an investigator who was blind to the animal grouping and carried out by normalizing individual peak areas as the percentage of total fatty acids.

2.3. Penetrating Arteriole Occlusion (PAO)

Mice were deeply anesthetized with 5% isoflurane, and then maintained at 2.5%, in oxygen with the anesthesia machine (RWD Life Science Co., Ltd., Shenzhen, China). To develop a model of penetrating arteriole occlusion (PAO) using two-photon microscopy, a thinned-skull window was prepared. Briefly, after the animal was placed in a stereotaxic apparatus, an incision with a sterile scalpel was made through the middle scalp skin of the mouse, and the skull bone was exposed by scraping away the periosteum. A 2×2 mm^2 region thinned-skull window was made over the left somatosensory cortex, which greatly minimizes disruption of the intracranial milieu. To image the vascular structure, the blood was labeled by intravenous injection of fluorescein isothiocyantate-dextran (FITC, 2000 kDa, FITC-d2000, 1.5% in saline, Sigma-Aldrich, Saint Louis, MO, USA) through the tail vein. To generate the PAO by damaging the endothelium of targeted vessels, intensively focused femtosecond laser pulses with a Ti:Sapphire laser (Coherent Chameleon Ultra II, CA) was used as a light source tuned at 800 nm with 140 fs pulse width and 80 MHz repetition rate [25,26,37]. The vascular injury was induced using the point bleach mode. The 6 points (one second per point) were focused on the two edges of the lumen of the target vessel in the same plane. To minimize possible collateral damage or avoid bleeding, the endothelium of the target vessel was damaged with an 800-nm laser, whose intensity (Max. power 3.5 W) was controlled by setting the electro-optic modulator (EOM) at 30% (roughly 1.05 W at the plane). In most cases, this injury triggered the natural clotting cascade, leading to localized clotting in the vessel. If the occlusion did not occur, we gradually increased the intensity by ~10% each time until the minimum power necessary to trigger clot formation in the target vessel was reached. The irradiation was repeated in case of recanalization (within 3 h after the injury). If vascular rupture occurred, the animal was excluded from the study. Overall, the incidence of vascular rupture is 4% (3 out of a total of 75 mice). Persistent occlusion was confirmed at 24 h by visual inspection and two-photon imaging. No death occurred in the PAO experiment.

2.4. In Vivo two-Photon Microscopy Imaging

To develop the model of PAO and evaluate the dynamic evolution of microcirculation and propidium iodide (PI)-staining after PAO, a Leica NA 0.95 and the 25× magnification water-immersion objectives were used. All images were acquired by using two-channel NDD detection with emission filter 525/50 nm and 585/40 nm under the same imaging parameters (laser power, photomultiplier tube voltage and gain) on a TCS SP5 MP System (Leica Microsystems, Mannheim, Germany). The person who performed image acquisition and image analysis was blind to the experiment.

2.5. The Volume of Blood Flux

The flux of the vessel of interest (F) was evaluated as described previously [37]. Briefly, the diameter of the penetrating arteriole of interest (d) was determined from at least 20 movie frames located in the surface segment of the penetrating arteriole. We used line scan to measure the centerline red blood cell (RBC) velocity (v) in individual vessels of interest at a line rate of 700 Hz. The centerline RBC velocity was automatically calculated with the Leica LAS AF 2.5 software. The average centerline RBC velocity for 30 s was referred to as the RBC speed of the vessels of interest. The flux of the vessel of interest (F) is equal to π/8 v (0) d^2. The measurement was performed by an investigator who was blind to the experiment.

Please cite this article as: Luo C, et al, Enriched Brain Omega-3 Polyunsaturated Fatty Acids Confer Neuroprotection against Microinfarction, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.05.028