Experimental Neurology 272 (2015) 170–180

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Dietary supplementation with omega-3 polyunsaturated fatty acids robustly promotes neurovascular restorative dynamics and improves neurological functions after stroke

Wenting Zhang [a,b,*], Hailian Wang [a], Hui Zhang [a], Rehana K. Leak [c], Yejie Shi [b,d], Xiaoming Hu [a,b,d], Yanqin Gao [a,b], Jun Chen [a,b,d,**]

[a] State Key Laboratory of Medical Neurobiology and Institute of Brain Sciences, and Departments of Anesthesiology and Neurology of Huashan Hospital, Fudan University, Shanghai 200032, China
[b] Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA
[c] Division of Pharmaceutical Sciences, Mylan School of Pharmacy, Duquesne University, Pittsburgh, PA 15282, USA
[d] Geriatric Research, Education and Clinical Center, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA 15261, USA

ARTICLE INFO

Article history:
Received 2 February 2015
Revised 2 March 2015
Accepted 6 March 2015
Available online 12 March 2015

Keywords:
Docosahexaenoic acid
Eicosapentaenoic acid
Revascularization
Angiogenesis
Neurogenesis
Oligodendrogenesis
Angiopoietin 1
Oligodendrocyte precursor cell

ABSTRACT

Stroke is a devastating neurological disease with no satisfactory therapies to preserve long-term neurological function, perhaps due to the sole emphasis on neuronal survival in most preclinical studies. Recent studies have revealed the importance of protecting multiple cell types in the injured brain, such as oligodendrocytes and components of the neurovascular unit, before long-lasting recovery of function can be achieved. For example, revascularization in the ischemic penumbra is critical to provide various neurotrophic factors that enhance the survival and activity of neurons and other progenitor cells, such as oligodendrocyte precursor cells. In the present study, we hypothesized that chronic dietary supplementation with fish oil promotes post-stroke angiogenesis, neurogenesis, and oligodendrogenesis, thereby leading to long-term functional improvements. Mice received dietary supplementation with n-3 PUFA-enriched fish oil for three months before and up to one month after stroke. As expected, dietary n-3 PUFAs significantly increased levels of n-3 PUFAs in the brain and improved long-term behavioral outcomes after cerebral ischemia. n-3 PUFAs also robustly improved revascularization and angiogenesis and boosted the survival of NeuN/BrdU labeled newborn neurons up to 35 days after stroke injury. Furthermore, these pro-neurogenic effects were accompanied by robust oligodendrogenesis. Thus, this is the first study to demonstrate that chronic dietary intake of n-3 PUFAs is an effective prophylactic measure not only to protect against ischemic injury for the long term but also to actively promote neurovascular restorative dynamics and brain repair.

Introduction

Ischemic stroke is the leading cause of adult disability worldwide (Demaerschalk et al., 2010). Indeed, more than 50% of its survivors suffer from long-lasting debilitation (Wiltrout et al., 2007). Tissue plasminogen activator (tPA) is the only FDA-approved therapy for stroke victims. However, clinical use of tPA is severely limited by its short temporal window of application (B. Zhang et al., 2011). Finding alternative therapies that are safe to administer as long-term prophylactic measures is therefore a matter of urgency in stroke research. Although many previous studies have shown that the adult brain has the ability to try and repair itself in response to ischemic insults, there are no safe, effective therapies that boost these endogenous repair mechanisms and thereby prevent stroke-induced neurological deficits. Thus, neurorestorative therapies that promote cerebral brain repair and neurological recovery post-ischemia have gained increasing attention in recent years (Hermann and Chopp, 2012; X. Liu et al., 2014; McLaughlin and Gidday, 2013).

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; Ang, angiopoietin; BrdU, 5-bromo-2-deoxyuridine; DAPI, 4,6-diamidino-2-phenylindole; DCX, doublecortin; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MBP, myelin basic protein; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MMP, matrix metalloproteinase; NPC, neural precursor cell; OPC, oligodendrocyte precursor cell; PDGF, platelet-derived growth factor; PUFA, polyunsaturated fatty acid; PVDF, polyvinylidene fluoride; rCBF, regional cerebral blood flow; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SVZ, subventricular zone; tMCAO, transient middle cerebral artery occlusion; VEGF, vascular endothelial growth factor.

* Correspondence to: W. Zhang, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China. Tel.: +86 21 54237395.
** Correspondence to: J. Chen, Center of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, S507 Biomedical Science Tower, 3500 Terrace Street, Pittsburgh, PA 15213, USA. Tel.: +1 412 648 1263.
E-mail addresses: wtzhang@fudan.edu.cn (W. Zhang), chenj2@upmc.edu (J. Chen).

http://dx.doi.org/10.1016/j.expneurol.2015.03.005
0014-4886/

One candidate that may possess neurorestorative properties is the administration of omega-3 polyunsaturated fatty acids (n-3 PUFAs), a major component of dietary fish oil. n-3 PUFAs are widely known for their critical role in neurodevelopment (Harris and Baack, 2015). Excessive consumption of n-6 PUFAs relative to n-3 PUFAs is thought to contribute to the higher incidence of stroke in Westernized nations (Simopoulos, 2002). In contrast, high levels of n-3 PUFAs are known to protect against ischemic brain damage in multiple animal models (Wang et al., 2014; Zhang et al., 2014; Zhang et al., 2010). However, the underlying mechanisms are not yet well-established. Multiple mechanisms have been proposed to mediate neuroprotection by n-3 PUFAs, including the activation of pro-survival signaling cascades in neurons, blunting of microglia-mediated inflammatory responses (Hu et al., 2014; Zhang et al., 2010), and suppression of oxidative stress (Begum et al., 2013; Zhang et al., 2014). The beneficial effects of n-3 PUFAs have been confirmed in *fat-1* transgenic mice expressing the *C. elegans fat-1* gene, which encodes an n-3 fatty acid desaturase that converts endogenous n-6 PUFAs into n-3 PUFAs. Our previous work has shown that overproduction of n-3 PUFAs in *fat-1* transgenic mice enhances angiogenesis, neurogenesis, and oligodendrogenesis following cerebral ischemia (Hu et al., 2013; Wang et al., 2014). However, dietary supplementation with n-3 PUFAs is more clinically relevant and whether it can promote brain repair and long-term recovery of neurological function is not yet known.

In the present study we tested the hypothesis that dietary supplementation with n-3 PUFAs would promote revascularization, neurogenesis, and oligodendrogenesis after stroke, thereby providing long-term neurobehavioral protection. Revascularization is one of the most effective endogenous repair mechanisms in the brain after stroke injury and illustrates the importance of a patent vascular network (Badaut and Bix, 2014; Li et al., 2014). Revascularization not only consists of reperfusion of pre-existing vasculature in the early phase after stroke but also consists of a surge in angiogenesis through endothelial cell proliferation in late stages (J. Liu et al., 2014). Post-stroke angiogenesis increases tissue perfusion with nutrients and oxygen while newly generated endothelial cells release a plethora of neurotrophic factors, thereby supporting the survival of neurons, oligodendrocytes, and their progenitor cells, and accelerating long-term recovery of neurological function (An et al., 2014; Merson and Bourne, 2014). In the past several decades, adult neurogenesis has been demonstrated in the subventricular zone (SVZ) of the lateral ventricle and the subgranular layer of the dentate gyrus. Neurogenesis can occur in response to cerebral ischemia and is characterized by the migration of SVZ-derived neural precursor cells along the blood vessels to the site of ischemic lesion (Ruan et al., 2014; Zhang et al., 2009). However, only a small proportion of these neural precursor cells survive and differentiate into mature neurons. Oligodendrocytes, the myelin forming cells of the central nervous system, are also sensitive to cerebral ischemia (Zhang et al., 2013). Stroke induces the loss of oligodendrocytes and elicits disruption of myelin, which impair axonal conductivity and exacerbate functional outcomes (Zhang et al., 2013). Oligodendrocyte precursor cells (OPCs) are known to differentiate into mature oligodendrocytes and form myelin sheaths for sprouting axons during brain repair, an important prerequisite for recovery of neurological function (Misumi et al., 2013; R.L. Zhang et al., 2011). Hence, finding therapies that can safely boost these endogenous repair processes would greatly facilitate the clinical treatment of stroke victims. It would also benefit future stroke victims if such therapies could be administered prophylactically for the long term as a preventative measure. To accomplish this goal, n-3 PUFAs were administered through dietary intake of fish oil for three months before and up to one month after stroke injury in the present study.

Here we show that chronic dietary administration of n-3 PUFAs robustly protects the adult brain against focal cerebral ischemia up to 35 days post injury. Furthermore, n-3 PUFAs significantly enhanced post-stroke angiogenesis, neurogenesis, and oligodendrogenesis. Thus, oral administration of n-3 PUFA-enriched fish oil is a promising preventative measure with the capacity to improve natural brain repair processes and facilitate long-term recovery of neurological function.

Material and methods

Dietary supplementation with fish oil

All experimental procedures were approved by the Animal Care and Use Committee at Fudan University. Four-week-old male C57BL/6J mice (Laboratory Animal LLC, Shanghai, China) were either fed a regular laboratory rodent diet with an inherently low n-3 PUFA concentration (0.5%) or the same diet supplemented with n-3 PUFAs (docosahexaenoic and eicosapentaenoic acids, triple strength n-3 fish oil, Puritan's Pride, Oakdale, NY, USA; final n-3 PUFA concentration 4%) for 3 months before exposure to transient cerebral ischemia and up to 35 days after ischemia until sacrifice.

Transient focal cerebral ischemia model

Transient focal cerebral ischemia was induced in adult male mice (4 months old, 25–30 g) by intraluminal occlusion of the left middle cerebral artery (MCA) for 60 min, as described previously (Wang et al., 2014). Experimental procedures were performed following the Stroke Therapy Academic Industry Roundtable (STAIR) guidelines (Fisher et al., 2009). Mice were anesthetized with 3% isoflurane vaporized in 30% O_2/70%N_2 until they were unresponsive to the tail pinch test. Animals were then fitted with a nose cone blowing 1.5% isoflurane for anesthesia maintenance. A monofilament (7-0) with a silicone-coated tip was introduced into the common carotid artery, advanced to the origin of the MCA, and left in place for 60 min. Rectal temperature was maintained at 37 ± 0.5 °C during surgery with a temperature-controlled heating pad. Arterial blood gases were analyzed at 15 min after the onset of ischemia and 15 min after reperfusion. Regional cerebral blood flow (rCBF) was measured using laser speckle during the entire procedure. Animals that did not show an rCBF reduction of at least 75% of the baseline levels or that died after ischemia induction were excluded from further experimentation. Sham-operated mice underwent the same anesthesia and surgical procedures without MCA occlusion (MCAO).

Laser speckle imaging

Animals were anesthetized with 1.5% isoflurane. A midline incision was made on the scalp, and tissues were removed with a scalpel to expose the skull surface. All procedures were performed using sterile technique. Laser speckle images (696 × 512 pixels) were acquired at 23 fps (exposure time T = 5 ms) by the laser speckle imaging system (Dolphin BioTech Ltd., Shanghai, China) with a laser diode (780 nm; Dolphin BioTech Ltd., Shanghai, China) placed over the skull (Miao et al., 2010). Briefly, 200 consecutive frames of speckle images were recorded per trial. Image processing took place off-line with MATLAB software (Mathworks Co., Ltd., USA). Raw speckle images were processed by the random process estimator (RPE) method after registration to obtain the contrast image. Changes in cerebral blood flow and the ischemic area were calculated by MATLAB software.

Measurement of infarct volume

Fourty-eight hours after MCAO, TTC staining was performed by a blinded investigator as we described previously (Stetler et al., 2008). Briefly, mice were anesthetized with 3% isoflurane and sacrificed. Brains were harvested and the forebrain was sliced into seven 1-mm-thick coronal sections. Sections were then stained with 2% 2,3,5--triphenyltetrazolium chloride (TTC) in saline at pH 7.4 (37 ± 0.5 °C) for 20 min, and fixed in 4% paraformaldehyde in PBS (pH 7.4) for 30 min before photography. Infarct volume was determined using the