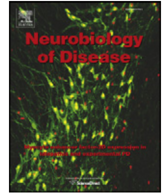
Neurobiology of Disease 91 (2016) 37–46

Contents lists available at ScienceDirect

Neurobiology of Disease

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



Omega-3 polyunsaturated fatty acids mitigate blood–brain barrier disruption after hypoxic–ischemic brain injury

Wenting Zhang [a,c,d,1], Hui Zhang [a,b,1], Hongfeng Mu [a], Wen Zhu [c,d], Xiaoyan Jiang [a,c,d], Xiaoming Hu [a,c,d], Yejie Shi [c,d], Rehana K. Leak [e], Qiang Dong [a,b], Jun Chen [a,c,d,f,⁎], Yanqin Gao [a,c,d,⁎⁎]

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

ARTICLE INFO

Article history:
Received 6 November 2015
Revised 11 February 2016
Accepted 23 February 2016
Available online 24 February 2016

Keywords:
Hypoxia/ischemia
Blood brain barrier
MMP-9
MMP-2
Tight junction

ABSTRACT

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been shown to protect the neonatal brain against hypoxic/ischemic (H/I) injury. However, the mechanism of n-3 PUFA-afforded neuroprotection is not well understood. One major determinant of H/I vulnerability is the permeability of the blood–brain barrier (BBB). Therefore, we examined the effects of n-3 PUFAs on BBB integrity after neonatal H/I. Female rats were fed a diet with or without n-3 PUFA enrichment from day 2 of pregnancy to 14 days after parturition. H/I was introduced in 7 day-old offspring. We observed relatively rapid BBB penetration of the small molecule cadaverine (640 Da) at 4 h post-H/I and a delayed penetration of larger dextrans (3 kD–40 kD) 24–48 h after injury. Surprisingly, the neonatal BBB was impermeable to Evans Blue or 70 kD dextran leakage for up to 48 h post-H/I, despite evidence of IgG extravasation at this time. As expected, n-3 PUFAs ameliorated H/I-induced BBB damage, as shown by reductions in tracer efflux and IgG extravasation, preservation of BBB ultrastructure, and enhanced tight junction protein expression. Furthermore, n-3 PUFAs prevented the elevation in matrix metalloproteinase (MMP) activity in the brain and blood after H/I. Thus, n-3 PUFAs may protect neonates against BBB damage by blunting MMPs activation after H/I.

Published by Elsevier Inc.

1. Introduction

Neonatal hypoxic–ischemic (H/I) brain injury is a catastrophic event with high morbidity and mortality. H/I injury may lead to long-term, irreversible neurological and cognitive deficits, including cerebral palsy, epilepsy, and neurodevelopmental disorders. To date, hypothermia is the only therapy showing promise in alleviating functional deficits in H/I patients (Srinivasakumar et al., 2013). Therefore, investigations of the pathological mechanisms underlying neonatal H/I brain injury and the identification of novel, safe therapies for neonates are urgently needed.

The blood brain barrier (BBB) is a large, dynamic structure that is evolutionarily designed to protect the vulnerable brain against blood-borne substances. The BBB is formed early in development and is essential for establishing a stable favorable microenvironment and facilitating nutritional support for cells in the central nervous system during brain maturation. The BBB is comprised of cerebral blood endothelial cells, pericytes, astrocytes, and highly restrictive tight junctions, all of which work in unison to form a robust physical barrier at the dynamic interface between blood, cerebrospinal fluid, and brain. Disruption of this barrier is known to lead to abnormalities in development and neurological function (Ek et al., 2015). However, the differential impact of ischemic injury on the neonatal versus adult BBB remains poorly understood. Early studies reported that the BBB in the developing brain is more vulnerable to ischemic challenges than in the mature brain (Muramatsu et al., 1997). However, recent evidence suggests that the BBB in neonates exhibits greater integrity after H/I injury than in the adult

Abbreviations: BBB, blood-brain barrier; Col-IV, collagen IV; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EB, evans blue; FcRn, neonatal Fc receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H/I, hypoxic/ischemic; LRP1, low-density lipoprotein receptor-related protein 1; MMP, matrix metalloproteinase; PUFA, polyunsaturated fatty acids; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TJ, tight junction; vWF, von Willebrand factor.

⁎ 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.
⁎⁎ Correspondence to: Y. Gao, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China.
E-mail addresses: chenj2@upmc.edu (J. Chen), yqgao@shmu.edu.cn (Y. Gao).

[1] These authors contributed equally to this work.

Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.02.020
0969-9961/Published by Elsevier Inc.

(Fernandez-Lopez et al., 2012). In both neonatal and adult models of stroke, BBB preservation is known to protect the brain against ischemic insults (Chen et al., 2009; Krueger et al., 2015; Li et al., 2013b). Thus, it is important to identify therapies that will preserve BBB integrity in the face of ischemic insults in both the developing and mature brain.

n-3 polyunsaturated fatty acids (PUFAs) are neurotrophic factors known for their beneficial roles in neurodevelopment. Previous research has shown that high levels of n-3 PUFAs protect both the adult and neonatal brain against ischemic brain damage via multiple mechanisms, including suppression of inflammatory responses and oxidative stress, enhancement of neurovascular unit reconstruction, and promotion of oligodendrogenesis (Chang et al., 2013; Chen et al., 2014; Hong et al., 2014; Wang et al., 2014; Zhang et al., 2014; Zhang et al., 2015). Recently, Hong et al. suggested that docosahexaenoic acid (DHA), the major n-3 PUFA in the brain, protects against BBB disruption after focal cerebral ischemia in adult rats (Hong et al., 2015). However, it is still unknown whether n-3 PUFAs can preserve BBB integrity following neonatal H/I brain injury.

Matrix metalloproteinases (MMPs) are a family of proteases that participate in physiological and pathophysiological processes, including at the BBB interface. Once activated, MMPs disrupt the BBB by degrading tight junctions and basal lamina proteins, leading to BBB leakage and brain edema. Early application of GM6001, a broad spectrum MMP inhibitor, soon after neonatal H/I brain damage reduces tight junction protein degradation and brain edema, thereby preserving the integrity of the young BBB (Chen et al., 2009). Furthermore, knockout of the MMP-9 gene significantly decreases IgG accumulation in the parenchyma 24 h after neonatal H/I (Svedin et al., 2007), suggesting that MMP-9 contributes to the early BBB opening (Moretti et al., 2015). Therefore, the present study characterized the impact of n-3 PUFAs on MMP protein and activity levels in both the plasma and the brain.

In the present study, we demonstrate for the first time that n-3 PUFA treatment protects the integrity of the neonatal BBB against H/I injury. Furthermore, our results support the view that inhibition of MMP production and activity might mediate the preservation of the BBB after n-3 PUFA treatment.

2. Material and methods

2.1. n-3 PUFA dietary enrichment and model of neonatal H/I brain injury

Animal experiments were approved by the Institutional Animal Care and Use Committee at Fudan University, and all procedures were strictly conformed to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines. Timed pregnant female Sprague–Dawley rats (Shanghai SLAC Laboratory Animal Co. Ltd. Shanghai, China) were randomly assigned to be fed a regular laboratory rodent diet with an inherently low n-3 PUFAs concentration (0.5%; N3L) or the same diet supplemented with n-3 PUFAs [N3H; DHA and eicosapentaenoic acid (EPA), Puritan's Pride, Oakdale, USA; final n-3 PUFAs concentration 1.5%] from the second day of pregnancy to 14 days after parturition. DHA and EPA contents were 60 and 90 mg, respectively, in every gram of N3H diet. Seven day-old rat pups were then subjected to H/I brain injury as previously described (Zhang et al., 2010). Briefly, the left common carotid artery was ligated under anesthesia with 3% isoflurane. After a 1.5-h recovery period, the pups were placed in a chamber containing a humidified atmosphere of 8% O_2/92% N_2 for 2.5 h and then returned to their dams. The overall mortality rate in the N3L H/I group was 8.4%, whereas it was 1.3% in the N3H H/I group. The majority of death in either groups occurred during or shortly after the hypoxic procedure, the dead animals were excluded from any further experiments or data analysis. In the sham surgery group, a ligature was placed loosely around the left common carotid artery but not ligated, and the pups were not subjected to hypoxia.

2.2. Brain water content measurement

Animals were sacrificed under deep anesthesia with 3% isoflurane at 48 h after H/I. The brain was harvested and dissected along the sagittal fissure into ipsilateral and contralateral hemispheres. Total wet weight was measured immediately and the brain was then heated to 100 °C in a drying oven for 72 h before measuring the dry weight (wet and dry weights were accurate to 0.1 mg). The percentage brain water content in each hemisphere was calculated with the following equation: ((wet weight − dry weight)/wet weight) × 100%.

2.3. Intravenous injection, detection, and quantification of tracers

Cadaverine Alexa-488 (640 Da; 200 μg/pup, Invitrogen, Waltham, USA) or dextran of various molecular weights (3 kD, 40 kD and 70 kD) was injected intravenously 1, 21, or 45 h post H/I. Animals were sacrificed 3 h following tracer infusions, perfused, and fixed with paraformaldehyde. Brains were removed and cut into 25 μm slices. Coronal brain sections were stained with RECA-1 (1:1000, AbD Serotec, Kidlington, UK) to visualize the cerebrovascular system. Fluorescence images were captured and the distribution of tracers was calculated using ImageJ software by a blinded observer.

2.4. Evans blue infusions

BBB permeability was determined by measuring Evans blue (EB) extravasation. A solution of 2% EB dye (3 mL/kg, Sigma Aldrich, St. Louis, USA) was slowly administered intravenously at 1, 21 and 45 h after reperfusion onset. Three hours following infusion, animals were perfused with 200 mL saline and decapitated. The brain was dissected into two hemispheres and soaked in methanamide separately for 48 h. To pellet the brain tissue, the sample was centrifuged for 30 min at 14,000 rpm. The absorption of the supernatant was measured at 632 nm with a spectrophotometer (Bio-Rad, Hercules, USA). The EB content was calculated as μg/g of brain tissue using a standardized curve by a blinded observer.

2.5. Measurement of endogenous immunoglobulin G (IgG) extravasation

The area of extravasation of endogenous IgG molecules was determined by immunohistochemical staining for rat IgGs on 25 μm-thick free-floating coronal brain sections. Sections were blocked with 5% goat serum in phosphate-buffered saline with 0.1% Triton-X 100 for 1 h, followed by a 2-h incubation in DyLight™ 488-conjugated goat-anti-rat IgG antibody (1:1000; Jackson ImmunoResearch Laboratories, West Grove, USA) at room temperature. The IgG-positive area was calculated by a blinded observer using ImageJ software. Four animals were analyzed in each group.

2.6. Transmission electron microscopy

BBB ultrastructure was detected by transmission electron microscopy at 48 h after ischemia. Proximal middle cerebral artery cortical tissues were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 12 h and 1% osmium tetroxide for 1 h. After dehydration in an alcohol series, tissues were embedded in 618# resin. Ultrathin sections were prepared using a Reichert ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined with a Philips CM120 electron microscope (FEI, Hillsboro, USA) at 80kv.

2.7. Western blotting

Western blots were performed using the standard SDS-PAGE method as previously described (Zhang et al., 2015). Briefly, PVDF membranes were blocked in 5% non-fat dry milk in Tris-buffered saline with Tween (TBST; 10 mM Tris–HCl, 150 mM NaCl, and 0.1% Tween)

Download English Version:

https://daneshyari.com/en/article/6021371

Download Persian Version:

https://daneshyari.com/article/6021371

Daneshyari.com