Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 38 (2016) 41–49

Journal of Nutritional Biochemistry

Folic acid deficiency increases brain cell injury *via* autophagy enhancement after focal cerebral ischemia☆,☆☆

Yaqian Zhao, Guowei Huang, Shuang Chen, Yun Gou, Zhiping Dong, Xumei Zhang*

Department of Nutrition and Food Hygiene, School of Public Health, Tianjin Medical University, Tianjin, China

Received 4 April 2016; received in revised form 9 June 2016; accepted 10 August 2016

Abstract

Folic acid (FA) deficiency is not only associated with an increased risk of ischemic stroke, but also with increased oxidative DNA damage and brain injury after cerebral ischemia–reperfusion. However, the cellular and molecular mechanisms underlying FA deficiency-associated neuropathogenesis are not completely understood. In the present study, we tested the hypothesis that neuronal autophagy in focal cerebral ischemia rats may be involved in the mechanisms of FA deficiency-induced injury to neuronal cells. The results demonstrated that, accompanied by obvious neuron damage, the expression of the autophagic markers LC3 and Beclin-1, and the formation of 8-OHdG (a marker of oxidative stress to DNA) and autophagosomes were significantly increased in the brain cortex after ischemia–reperfusion. FA deficiency further induced neuronal cell death, and significantly increased the formation of autophagosomes and the expression of LC3 and Beclin-1 in NeuN-positive cell bodies after ischemia–reperfusion. The elevated level of 8-OHdG was also observed in the ischemic cortex of FA deficiency-treated animals. Conversely, the neuronal cell injury, autophagosome accumulation and the effects of LC3 and Beclin1 overexpression caused by FA deficiency were partially blocked by an autophagic inhibitor 3-methyladenine. These results suggest that FA deficiency progresses autophagic activation and aggravates the damage in rat brain cortex following focal cerebral ischemia–reperfusion. The oxidative injury may be involved in cell morphological damage and autophagy alteration caused by FA deficiency.
© 2016 Elsevier Inc. All rights reserved.

Keywords: Folic acid deficiency; Autophagy; Cerebral ischemia–reperfusion; Cortex; Oxidative stress

1. Introduction

A stroke is characterized by cerebral ischemia or hemorrhagic injury. It is associated with high morbidity and mortality, and is one of the most serious medical conditions that can harm human health [1]. Numerous research accounts show that cerebral ischemia–reperfusion causes neuronal injury, which seriously affects the functional activities of the brain [2]. Multiple pathophysiological mechanisms have been observed in ischemic stroke/cerebral ischemia including cellular energy metabolism disturbance, excitatory amino acid neurotoxicity, intracellular calcium overload, oxygen free radical and nitric oxide damage, and inflammatory responses [3]. However, our understanding of definitive, underlying mechanisms remains incomplete to date. Uncovering the mechanisms of brain cell injury might lead to the development of an effective treatment for ischemic stroke.

Folic acid (FA) is an essential nutrient component of normal human diet involved in numerous metabolic reactions, for example, DNA and RNA biosynthesis and amino acid interconversions [4]. The importance of FA in the nervous system was also demonstrated in many other neurological disorders, including neural tube defects in fetuses and Alzheimer's disease in the elderly [5]. Previous studies showed that low levels of FA are associated with ischemic stroke; FA supplementation as a primary prevention could reduce the risk of stroke by 18% [6]. In addition to augmenting the risk for vascular events, low FA may directly increase the susceptibility of brain injury, damaging neurons, in ischemic stroke [7]. Our previous study also found that FA enhances the stimulation of Notch signaling and hippocampal neurogenesis in adult brains, and lessens the impairment of cognitive function that occurs after experimental stroke [8]. The present study will provide new insight into how FA deficiency causes brain cell injury after ischemic stroke.

Autophagy is a highly conserved cell degradation pathway during which the cytoplasm, organelles and/or other substances are enveloped by double-membrane vesicles called autophagosomes, which fuse with lysosomes to form autophagolysosomes, which then have their contents degraded [9]. Some previous studies have reported that

☆ Chemical compounds studied in this article: Folic acid (PubChem CID: 6037); 3-methyladenine (PubChem CID: 1673).

☆☆ Funding sources: This work was supported by the grants from the National Natural Science Foundation of China (No. 81373003) and China Postdoctoral Science Foundation (No. 2014M550148).

* Corresponding author at: Department of Nutrition and Food Hygiene, School of Public Health, Tianjin Medical University, No. 22 Qixiangtai Road, Heping District, Tianjin 300070, China. Tel.: +86 22 83336615; fax: +86 22 83336606.

E-mail address: zhangxumei@tmu.edu.cn (X. Zhang).

http://dx.doi.org/10.1016/j.jnutbio.2016.08.009
0955-2863/© 2016 Elsevier Inc. All rights reserved.

autophagy is induced in cerebral ischemia in various animal models, including focal or global cerebral ischemia, or hypoxia–ischemia models in rats and mice [10–12]. Mild to moderate ischemia can lead to the occurrence of autophagy and apoptosis of neurons, shown in a model of ischemic cell injury [13]. Autophagy activation offers remarkable tolerance against subsequent, fatal ischemic insult as demonstrated in an animal model of cerebral ischemic preconditioning [14]. As aforementioned, both FA deficiency and activation of autophagy are closely related to the pathological process of ischemic stroke. However, whether and how autophagy is involved in FA deficiency-induced neural cell injury after brain ischemia has not been reported until now. Here, we tested the hypothesis that FA deficiency may promote neuronal autophagy and exert the neurotoxic effect in a focal cerebral ischemic model.

Accumulating lines of evidence suggest that oxidative stress plays an important role in mediating autophagy in various cell types and organs [15]. Activation of macroautophagy is part of the cellular response to oxidative stress, for removal of damaged components before further damage/aggregation occurs [16]. On the contrary, oxidative stress has been implicated in neuronal damage caused by FA deficiency [17]. Therefore, it is impossible that FA deficiency may alter autophagy levels by raising oxidative damage.

The present study was designed to elucidate the neurotoxic effect of FA deficiency in ischemic brains and to determine its underlying mechanisms. Here, we provide evidence that FA deficiency-triggered autophagy overactivation contributed to brain neuronal cell injury and describe whether it was accompanied by oxidative damage.

2. Materials and methods

2.1. Animals

One hundred, SPF grade, male Sprague Dawley rats, weighing 180–200 g [SCXK (Jing) 2012–0001], were provided by Peking Weitong Lihua Experimental Animal Technology Center (Beijing, China). The experiments described below were performed in compliance with institutional guidelines under approved protocols. Rats were randomly divided according to body weight into sham-operated control group (SHAM), middle cerebral artery occlusion–reperfusion group (MCAO), MCAO plus deficient FA diet group (MCAO+Free-FA), MCAO+Free-FA plus 3-methyl adenine group (MCAO+Free-FA+3MA) and MCAO plus 3-methyl adenine group (MCAO+3MA); 20 rats were in each group. In the SHAM and MCAO groups, the normal and FA-deficient diets (Keao Xieli Company, Beijing, China) were administered for 28 days prior to SHAM or MCAO operation. 3MA (5 mmol/L; 4 ml/kg; Sigma, St Louis, MO, USA) was administered by tail vein injection for 5 days prior to the SHAM or MCAO operation.

2.2. Surgical procedures

Rats were anesthetized with 10% chloral hydrate (3 ml/kg). MCAO model was induced by the modified Longa method [18]: The left external carotid artery was ligated by the suture line. A nylon thread was advanced by the left common carotid artery, through the left internal carotid artery and into the origin of the middle cerebral artery. The thread was pulled out 1 cm and cut off 1 h after the operation. Animals assigned to SHAM operation were treated similarly, except that the thread was not advanced to the origin of the middle cerebral artery. The rats were then allowed to recover from anesthesia and were sacrificed at 24 and 72 h after reperfusion for the following experiments.

A neurological score was assigned to each animal 10 min after waking up according to the Longa method [18]: no deficit=0; contralateral forelimb weakness=1; circling to contralateral side=2; partial paralysis on contralateral side=3; and no spontaneous motor activity=4. MCAO rats with neurological deficit scores of 1–3 were left for experiments.

2.3. Concentration of serum FA

Blood (1 ml) was respectively taken from the angular vein of rats in each group before intervention and surgical procedures, and centrifuged for 10 min at 3000 r/min to obtain the supernatant. The supernatant was then used to detect the concentration of serum FA using a FA detection kit (Abbott, Chicago, IL, USA).

2.4. Transmission electron microscopy

Three rats from each group were randomly assigned to have their brains taken 24 h after reperfusion. The cerebral cortex fragments were fixed with 2.5% glutaraldehyde solution overnight at 4°C, and postfixed with 1% osmic acid for 2 h. After gradient acetone dehydration, the samples were embedded in an Epon/Araldite mixture. Sections were then sliced and stained with uranyl acetate and lead citrate, and then were observed and photographed under a transmission electron microscope (HT-7700; Hitachi, Tokyo, Japan).

2.5. Preparation for paraffin section

Rats were deeply anesthetized 24 and 72 h after the MCAO operation [24 h for immunofluorescence and 72 h for hematoxylin and eosin (HE) and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) staining] with 10% chloral hydrate and were perfused with 0.9% saline solution followed by 4% phosphate-buffered paraformaldehyde (PFA). Left brains were quickly removed and further immersed in 4% PFA overnight. Then brain tissues were equilibrated in phosphate-buffered 30% sucrose, embedded in paraffin and cut into 6-μm coronal sections.

2.6. HE and TUNEL staining

Three rats of each group were separately assigned to HE and TUNEL staining. Sections of brain cortex embedded in paraffin were stained with HE for routine histological examinations and morphometric analyses under a light microscope (IX81; Olympus, Tokyo, Japan).

The TUNEL assay was performed using the *In Situ* Cell Death Detection Kit (Roche Company, Basal, Switzerland) as described previously [19]. Paraffin sections were deparaffinized and then treated with 3% hydrogen peroxide in methanol for 10 min. After, sections were treated with TdT-enzyme at 37°C for 1 h. Sections were then incubated with digoxigenin-conjugated antibodies at 37°C for 30 min. Finally, sections were colorized with DAB. Six sections of each rat brain were observed and photographed under a light microscope (IX81; Olympus). Image-Pro Plus 6.0 was used to count positive cells.

2.7. Immunofluorescence analyses

For immunofluorescence, sections were incubated with the primary antibody (anti-LC3 (1:400; Cell Signaling Technology, Boston, MA, USA), anti-Beclin-1 (1:200; Abcam, Cambridge, UK), anti-NeuN (1:1000; Abcam), anti-GFAP (1:100; Abcam) and anti-8-OHdG (1:500; Abcam) overnight at 4°C, followed by the corresponding secondary antibody, the goat anti-rabbit (1:100; Zhongshan Goldbridge Biotechnology, Beijing, China) or the goat anti-mouse (1:100; Zhongshan Goldbridge Biotechnology, Beijing, China), for 1 h at room temperature. The sections were then viewed under a fluorescence microscope (IX81; Olympus). DAPI was used to dye the nuclei before mounting. Image-Pro Plus 6.0 was used to count positive cells.

2.8. Immunoblotting analysis

Animals were sacrificed 24 h after reperfusion. Brain tissue extract was obtained from three rats of each group, and the protein concentrations in the supernatants were determined using the BCA protein assay system (Boster Biological technology Ltd., Wuhan, China) according to the Bradford method [20]. The samples were then analyzed by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose blotting membranes (Millipore, Bedford, MA, USA) was performed according to the wet electrical transfer method. The sheets were soaked in 5% skimmed milk at 37°C for 1 h, incubated with the primary antibody (anti-LC3, 1:1000; anti-Beclin-1, 1:500; and anti-β-actin, 1:10,000; Abcam) overnight at 4°C, and then incubated with the corresponding secondary antibody (goat anti-rabbit, 1: 10,000; Zhongshan Goldbridge Biotechnology, Beijing, China) for 1 h at room temperature. The membranes were then treated with Immobilon Western chemiluminescent HRP substrate (Millipore) for 2 min and then observed using the ChemiDoc XRS+ Imaging System (Bio-Rad, Hercules, CA, USA). Gray values of immunoreactive bands were analyzed by Image J 1.4.3.67. The ratios of LC3-II/LC3-I and Beclin-1/β-actin were used to analyze the expression of LC3 and Beclin-1, respectively.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 19.0 (IBM Corporation, Armonk, NY, USA). Data were expressed as mean±standard deviation ($\bar{x}\pm s$). Differences between means were determined by one-way analysis of variance followed by LSD multiple range tests. $P<.05$ was assumed statistically significant.

Download English Version:

https://daneshyari.com/en/article/8336504

Download Persian Version:

https://daneshyari.com/article/8336504

Daneshyari.com