Prostaglandins, Leukotrienes and Essential Fatty Acids 128 (2018) 11–20

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

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

Maternal dietary of n-3 polyunsaturated fatty acids affects the neurogenesis and neurochemical in female rat at weaning

Mimi Tang[a,b], Min Zhang[a,b], Lu Wang[a,c], Huande Li[a,*], Hualin Cai[a,*], Ruili Dang[d], Pei Jiang[d], Yiping Liu[a], Ying Xue[a,b], Yanqin Wu[a,b]

[a] Institute of Clinical Pharmacy & Pharmacology, Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, PR China
[b] School of Pharmaceutical Sciences, Central South University, Changsha, PR China
[c] Mental Health Institute of the Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, PR China
[d] Institute of Clinical Pharmacy, Jining First People's Hospital, Jining Medical University, Jining 272000, PR China

ARTICLE INFO

Keywords:
Polyunsaturated fatty acids (PUFAs)
Neurogenesis
Neurotransmitter system
Turnover rate
Between-metabolite ratios

ABSTRACT

Long-chain polyunsaturated fatty acids (LC-PUFAs) are rapidly accumulated in brain during pre- and neonatal life, which is important for the development and function of central nervous system. Deficiency of biologically important n-3 PUFA docosahexaenoic acid (C22:6n–3, DHA) is associated with impaired visual, attention and cognition, and would precipitate psychiatric symptoms. However, clinical studies of the potential mechanism on the effect of dietary DHA deficiency on neural development remain unclear. In addition, the effects of n-6 PUFAs and n-3 PUFAs ingestion on the dynamic process of the cell proliferation in neurogenesis of offspring were investigated using immunefluorescence. And GC-MS was used to determine the fatty acid content in the liver of offspring. To further investigate the neurochemical influence on maternal PUFAs levels, we assessed the functioning of various neurotransmitter systems including glutamatergic, dopaminergic, norepinephrinergic and serotoninergic systems in the brain of female rats at weaning by HPLC-MS/MS. Lastly, we analyzed the turnover rates and between-metabolite ratios (the ratios between metabolites of monoamine neurotransmitters) to seek potential links between the neurotransmitters and dietary fatty acids compositions. There were significant differences between the deficiency group and the control or supplementary group in liver fatty acids compositions, showing that n-3 PUFAs were largely replaced by n-6 PUFAs. The generation of n-3 PUFAs deficiency rats exhibited abnormal neurogenesis and neurochemical. Altered dopamine or norepinephrine transmission and between-metabolite ratios in brain areas may be a key neuronal mechanism that contributes to the potential detrimental effects of n-3 PUFAs deficiency for mental health.

1. Introduction

Long-chain polyunsaturated fatty acids (LC-PUFAs) are synthesized from two independent and nutritionally essential PUFAs, a-linolenic acid (C18:3n3, ALA) and linoleic acid (C18:2n6, LA) by chain elongation and desaturation [1]. Because mammals cannot synthesize n-3 or n-6 PUFAs de novo, they are entirely dependent on dietary sources to produce and maintain adequate concentrations in peripheral and central tissues [2]. Meanwhile, mammals have the enzymes to elongate the essential FA to biologically important n-3 and n-6 LC-PUFAs such as arachidonic acid (C20:4n–6, AA), eicosapentaenoic acid (C20:5n–3, EPA) and docosahexaenoic acid (C22:6n–3, DHA). LC-PUFAs are rapidly accumulated in brain during pre- and neonatal life and they are important for the development and function of central nervous system [3]. Decreased availability of DHA during development is associated with deficits in visual, attentional and cognitive function, and exhibited abnormal behavior (anxiety, aggression) and cognitive function [4,5]. Thus, the lipid requirements of young infants, particularly the requirements for the n–6 and n–3 fatty acids for brain growth and development, are currently an area of intense interest.

Neurogenesis in the dentate gyrus (DG) of the hippocampus occurs throughout the lifetime of humans and rodents, and is related to the functions of learning and memory [6]. The numbers of proliferating newborn neurons in the DG decreases with age [7]. Several previous reports have addressed the association between neurogenesis and tissue fatty acid compositions. Sakayori et al. found that AA and DHA have

* Corresponding authors.
E-mail addresses: tangmimi1989@163.com (M. Tang), csuzhangmin@163.com (M. Zhang), luwang112@163.com (L. Wang), lihuande1953@126.com (H. Li), ghostspecialist@163.com (H. Cai), ruilidang@gmail.com (R. Dang), jpoet89@126.com (P. Jiang), yipingliu1973cn@yahoo.com.cn (Y. Liu), xueying091@126.com (Y. Xue), wuyanqin1992@163.com (Y. Wu).

https://doi.org/10.1016/j.plefa.2017.11.001
Received 13 January 2017; Received in revised form 16 October 2017; Accepted 8 November 2017

Table 1
Fatty acid composition of the Experimental Diets.

Fatty acid	Content in diet (area percent)		
	Deficiency	Control	Supplementary
C16:0	6.61	11.18	9.96
C18:0	2.31	3.17	3.39
C20:0	ND	4.26	3.67
C18:1n9c	11.71	24.52	22.72
C18:2n6c	79.38	55.39	47.30
C20:5n3	ND	ND	7.32
C22:6n3	ND	ND	4.00
Other MUFA	ND	1.48	1.95

Diet fatty acid composition was determined by GC/MS using Supelco 37 Standard. ND: Not detected.

different effects on maintenance and differentiation of neural stem/progenitor cells using a neurosphere assay [8]. Administration of DHA for 7 weeks was reported to increase the number of newborn neurons in aged n-3 PUFAs-deficient rats in the third generation of diet-deficient breeding [9]. A recent study showed that the number of newborn neurons was correlated with DHA content in red blood cells, and the DHA may support the production and survival of newborn neurons [10]. However, the effects of n-6 PUFAs and n-3 PUFAs ingestion on the dynamic process of the cell proliferation in neurogenesis of offspring remain unclear.

The mechanisms of n-3 PUFAs deficiency in inducing neurodevelopment impairment are still unclear. One of possible assumptions may that it is related to its effects on multiple neurotransmitter systems, such us glutamatergic system, dopaminergic system, noradrenergic system and serotonergic system [11]. Dietary n-3 PUFAs supplementary was reported to restore dopamine (DA) metabolism and normalize brain DA level in mice [12]. On the contrary, n-3 fatty acid deficiency has been shown to alter densities of dopamine receptors [13]. Similarly, diet containing inadequate n-3 PUFAs was also reported to influence the levels of norepinephrine (NE) and serotonin (5-HT), or even alter the expressions of related receptors in certain brain areas [11]. However, the potential associations between neurotransmitters and its metabolites need further studying, not to mention the between-metabolite ratios. To further analyze how the development of central nervous systems is affected by the availability of dietary n-3 PUFAs, we designed this study to reveal the relationship between the turnover rates and between-metabolite ratios of neurotransmitters and dietary fatty acids composition. Previous findings have shown significant deficits of DHA in the prefrontal cortex of postmortem female, but not male, in patients with major depression [2]. Moreover, cross-sectional epidemiological surveys suggest that low dietary omega-3 fatty acid intake is associated with an increased risk for depression in females but not in males [14,15]. Gender differences might be involved in the pathophysiology of n-3 fatty acid deficiency induced neurodevelopment impairment [16]. Therefore, we focused on the changes of female offspring in the present study.

2. Methods

2.1. Animals

Sprague-Dawley rats were housed in groups in a temperature-controlled environment under a 12 h light-dark cycle with free access to food and water. This study was approved by the Animal Care & Use Committee of Central South University. All experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals (Chinese Council).

2.2. Experimental procedure and husbandry

Rats were randomly divided into three groups (n = 7) according to their diets (n-3 PUFAs content): Deficiency, Control and Supplementary. Breeding stock (Female, 210–230 g; Male proven breeders; The Experimental Animal Center of the Second Xiangya Hospital) maintained corresponding diets for two weeks before mating (Female 280–300 g) until the end of the experiment. One male rat was housed with two female rats per cage for three days at the time of mating. When the pups were born, the dams were kept individually with the litters. Two or three female pups were randomly selected from each litter for neurogenesis determination. After weaning, one or two female pups were randomly selected again from each litter for fatty acids composition and neurochemical analysis.

An AIN-93G (Trophic Animal Feed High-Tech Co., Ltd, China) was used to meet all current nutrient standards for rat pregnancy and growth [17]. The deficiency and supplementary diet were identical to the control diet except the oil formulation. The control diet in our experiment was formulated with soybean oil (70 g/kg), whereas the deficiency diet was prepared with safflower oil (70 g/kg) and the supplementary diet was prepared with fish oil (20 g/kg) and soybean oil (50 g/kg). Fatty acid composition of the diets is revealed in Table 1. After weaning (PND 21), female offspring were anesthetized with chloral hydrate, and the tissues were collected and stored at −80 ℃ for fatty acids and neurochemical analysis.

2.3. The neurogenesis in female rat offspring

Proliferating cells in the hippocampus were examined at postnatal (PND) 4, 8 and 15 days (detail process was shown in Fig. 1). To observe the dynamic process of the cell proliferation in offspring female rats, bromodeoxyuridine ((+)−5'-bromo-2'-deoxyuridine [BrdU]; Sigma-Aldrich, USA) in 0.9% sterile saline solution was injected intraperitoneally twice daily (at 12-h intervals) at dose of 50 mg/kg for 3 consecutive days [18]. After injection of BrdU, rats were sacrificed and brains were removed and fixed overnight in 4% paraformaldehyde. And then, tissues were cryoprotecter in 30% sucrose in PBS before embedding. Immunefluorescence of BrdU-labeled nuclei was measured using BrdU Assay kit (servicebio, China) according to the protocol.

2.4. FA composition assays

GC-MS analyses were carried out on Agilent 7890 A/5975 C, with

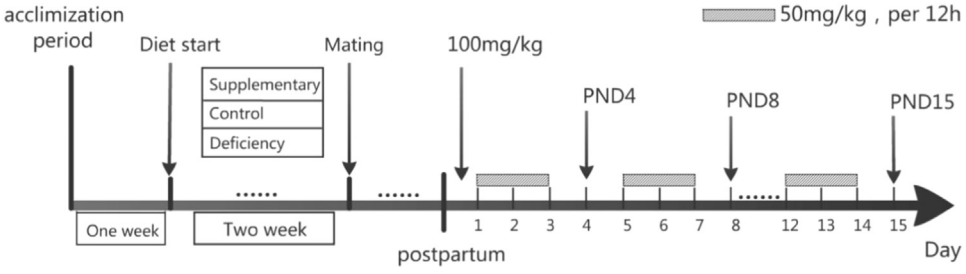

Fig. 1. Timeline of the experimental procedures.