Prostaglandins, Leukotrienes and Essential Fatty Acids 106 (2016) 27–37

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

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

Research article

Dietary docosahexaenoic acid alleviates autistic-like behaviors resulting from maternal immune activation in mice

Michael J. Weiser [c,*], Brittany Mucha [a], Heather Denheyer [a], Devon Atkinson [a], Norman Schanz [a], Evros Vassiliou [b], Robert H. Benno [a]

[a] William Paterson University, Dept. of Biology, Wayne, NJ, United States
[b] Kean University, Dept. of Biological Sciences, Union, NJ, United States
[c] DSM Nutritional Products, Human Nutrition and Health, Boulder, CO, United States

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form
30 October 2015
Accepted 30 October 2015

Keywords:
Development
Maternal immune activation
Omega-3 fatty acids
Social behavior
Pregnancy
Nutrition

ABSTRACT

The prevalence of autism spectrum disorders over the last several decades has risen at an alarming rate. Factors such as broadened clinical definitions and increased parental age only partially account for this precipitous increase, suggesting that recent changes in environmental factors may also be responsible. One such factor could be the dramatic decrease in consumption of anti-inflammatory dietary omega-3 (n-3) polyunsaturated fatty acids (PUFAs) relative to the amount of pro-inflammatory omega-6 (n-6) PUFAs and saturated fats in the Western diet. Docosahexaenoic acid (DHA) is the principle n-3 PUFA found in neural tissue and is important for optimal brain development, especially during late gestation when DHA rapidly and preferentially accumulates in the brain. In this study, we tested whether supplementation of a low n-3 PUFA diet with DHA throughout development could improve measures related to autism in a mouse model of maternal immune activation. We found that dietary DHA protected offspring from the deleterious effects of gestational exposure to the viral mimetic polyriboinosinic–polyribocytidilic acid on behavioral measures of autism and subsequent adulthood immune system reactivity. These data suggest that elevated dietary levels of DHA, especially during pregnancy and nursing, may help protect normal neurodevelopment from the potentially adverse consequences of environmental insults like maternal infection.

1. Introduction

Autism is a disabling neurodevelopmental disorder with clinically identifiable symptoms early in childhood that affects approximately one in 132 people globally and appears to be associated with complex genetic and environmental risk factors [1,2]. The core symptoms of autism include repetitive stereotypic behaviors, deficits in social interaction behaviors, and difficulties with verbal and non-verbal social communication. Recent studies have indicated a putative role for maternal immune activation (MIA) (such as viral or bacterial infection during gestation) as an environmental risk factor associated with neurodevelopmental disorders such as autism and schizophrenia [3,4]. Developed within the past decade, rodent models of prenatal infection have provided insights into the putative role of neuroinflammation in the development of schizophrenia [5,6] and autism spectrum disorders (ASD; [7]). A number of inflammatory agents including lipopolysaccharide (LPS; a component of gram-negative bacteria) and polyriboinosinic-polyribocytidilic acid (Poly I:C; a synthetic double stranded RNA) are used to mimic a prenatal bacterial or viral inflammatory event (respectively) at different time points during gestation. These models have produced data indicating that maternal inflammation alters aspects of neuronal function and behavior in rodents in a manner consistent with dysfunctions observed in schizophrenia and autism in humans [5,8,9]. For example, injection of Poly I:C during gestation impairs pre-pulse inhibition in the offspring when tested as adults in both rats and mice [10,11], a characteristic impairment typically observed in schizophrenic humans. Furthermore, injection of Poly I:C on day 12 of gestation in the C57BL/6 J mouse produces a decrement in social interaction, a hallmark phenotype of autism in humans [8].

While these studies are informative, statistical analysis shows that the inflammatory agent only accounts for a small amount of the variance reported in these studies, and that other factors including genes are involved. A recent study by Schwartzer *et al.*, [7]

Abbreviations: ASD, autism spectrum disorders; DHASCO, docosahexaenoic acid-rich single-cell oil; GD, gestational day; IL, interleukin; LPS, lipopolysaccharide; MIA, maternal immune activation; P, postnatal day; Poly I:C, polyriboinosinic-polyribocytidilic acid; RBC, red blood cell; TNFα, tumor necrosis alpha

* Corresponding author.
E-mail address: michael.weiser@dsm.com (M.J. Weiser).

http://dx.doi.org/10.1016/j.plefa.2015.10.005
0952-3278/

in which they used prenatal injections of Poly I:C in two strains of mice, C57BL/6 J and BTBR T+ tf/J (BTBR) demonstrated the value of a gene by environment approach. In this study they showed that the Poly I:C was effective in producing a more dramatic autistic phenotype in the BTBR mouse, a strain previously shown to have an autistic-like phenotype relative to the C57BL/6 J strain. However, the behavioral changes produced by prenatal exposure to Poly I:C in this study differ significantly from those observed by Malkova *et al.* [8]. These discrepancies led Schwartzer *et al.* to speculate that the effects of maternal poly I:C exposure on offspring are dependent on additional factors, including the environment.

One element accounting for the variability in these studies might be an inability of the mother to effectively deal with the inflammatory response produced by the poly I:C injection. Along these lines, the environmental factor of diet, which varies widely across laboratories, might affect inflammatory susceptibility and contribute to the observed variability. Differences in rodent chow are not typically considered as variables in experiments, unless researchers decide *a priori* to define diet as an independent variable. One important difference across diets is the amount of omega-3 (n-3) polyunsaturated fatty acids (PUFAs) that are present, as determined by the source of dietary fat. This fat source is typically corn or soybean oils which contain low amounts of n-3 PUFAs and high amounts of pro-inflammatory omega-6 (n-6) PUFAs, and not canola or flaxseed oils which are higher in n-3 PUFAs.

The n-3 PUFA docosahexaenoic acid (DHA; 22:6n-3) is an essential component of membrane phospholipids and is important for many biological systems throughout the body and across the lifespan, particularly during development. DHA rapidly and selectively accumulates in neural tissues during development accounting for over 95% of the n-3 PUFAs in the brain [12], and the dietary status of the mother directly affects the levels of DHA not only in her tissues, but also in the offspring by way of maternal transfer during gestation and lactation [13,14]. Importantly, vertebrates lack the necessary enzymes for *de novo* n-3 PUFA synthesis, and must obtain these essential fatty acids from the diet [15]. Unfortunately, humans are inefficient in producing DHA from its n-3 PUFA precursors, namely α-linolenic acid [16], and thus require dietary sources of preformed DHA such as fatty fish, or supplementation with oils derived from fish or microalgae to obtain optimal tissue levels of DHA [17–19].

Dietary n-3 PUFA deficiency during development in rodents leads to deficits in cognition, vision, and a wide array of behaviors [20]. For example, mice developmentally deprived of n-3 PUFAs have significantly impaired prepulse inhibition [21], and rats deficient in DHA throughout development exhibit an increase in depressive-like behavior immediately following the pubertal transition [22]. These data are corroborated by human evidence indicating a positive effect of DHA during pregnancy and/or infancy on a wide range of developmental measures including gestational length, visual acuity, attention, and reading ability [23–25]. A recent review by van Elst *et al.* [26] suggests that the considerable rise in autism in humans seems to parallel the change in our food sources resulting in a drastically increased ratio of n-6 to n-3 PUFAs in our diets. Using data from both clinical and experimental research van Elst *et al.* argue that the change in the ratio of n-6 to n-3 PUFAs especially during early life, may induce developmental changes in brain connectivity, synaptogenesis, cognition, and behavior that are directly related to ASD. Although the mechanism of action by which DHA deficiency might lead to altered brain development related to ASD is unclear, it has been shown that diets rich in DHA lead to a decrease in neuroinflammatory responses to a wide range of substances [27]. Since exposure to Poly I:C during development produces a neuroinflammatory response one might speculate that providing preformed DHA through the diet could inhibit the effect of prenatal exposure to poly I:C and decrease autistic-like behaviors in this model.

In this study, we sought to determine if supplementation of a low n-3 PUFA diet with DHA could affect the behavioral phenotype resulting from prenatal infection in mice. We provided an inflammatory insult (Poly I:C) at gestational day 12 in mice that had been maintained on diets either deficient or sufficient in DHA before mating and during gestation and lactation, and subsequently maintained the offspring on the respective maternal diet after weaning. We examined autistic-like behaviors previously correlated with *in utero* exposure to Poly I:C, and found that dietary DHA effectively rescued the autistic-like phenotype observed in the DHA-deficient offspring. Furthermore, dietary DHA throughout development buffered the heightened immune mediated responses in adulthood observed in the DHA-deficient offspring.

2. Materials and methods

2.1. Animals

A colony of C57BL/6 J mice, originally obtained from Jackson Laboratory, were bred and maintained in the vivarium at William Paterson University according to PHS guidelines for animal experiments. A separate group of 129 S1 mice, obtained from Jackson Laboratory were used as target mice in the social interaction phase of the experiment. All experimental procedures and protocols were reviewed and approved by the institutional animal care and use committee at William Paterson University. Animals were provided chow and water *ad libitum* and housed in polycarbonate cages in a temperature and humidity controlled environment on a 12 h:12 h light:dark cycle.

2.2. Diet composition

The DHA-deficient (low n-3 PUFA) and DHA-sufficient diet were prepared by Dyets, Inc. (Bethlehem, PA, USA) and were based on the AIN-93G formulation [28,29], with 7% total fat derived from a custom fat blend containing olive, hydrogenated coconut, and high-oleic safflower oils, and DHASCO (DSM Nutritional Products) which is a DHA-rich oil (min. 40% by wt.) derived from algae. Composition of the two diets is shown in Table 1. Each diet contained, as a percentage of total fatty acids (FAs), approximately 8% linoleic acid (18:2n6), and 0.4% α-linolenic acid (18:3n3), but differed in amount of DHA (22:6n3; 0% for deficient vs. 1% for sufficient) and therefore total n-3 fatty acids (0.40% vs. 1.43%) and n6:n3 ratio (20.77% vs. 5.71%).

2.3. Experimental design

At the time of weaning (21 days), female C57BL/6J mice were randomly assigned to two groups: one received the control diet (low n-3 PUFA, lacking DHA) and the other received a similar low n-3 PUFA base diet to which DHA was added (0.07% of the diet by weight; 1% of total fatty acids by weight). The animals were maintained on these diets throughout the remainder of the experiment. At approximately 60 days of age the experimental animals were timed mated (males and females are paired from 5 P.M. until 9 A.M. the next morning). Pregnancy was determined by the presence of a vaginal plug and significant weight gain over the next 12 days. On day 12.5 following finding of the vaginal plug, the animals from each dietary group were placed into one of two prenatal insult groups. One group received a 20 mg/kg intraperitoneal injection of the viral mimic Poly I:C (Sigma) dissolved in 0.9% sterile saline, while the second group received a 0.9% saline control injection. This injection protocol has been used successfully by