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Targeting inflammation in the preterm infant: The role of the omega-3 fatty acid docosahexaenoic acid

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ABSTRACT

Long-chain polyunsaturated fatty acids are critical for the normal growth and development of preterm infants. Interest in these compounds rests in their anti-inflammatory properties. Clinical conditions with an inflammatory component such as bronchopulmonary dysplasia, necrotising enterocolitis and sepsis are risks to the survival of these infants. Dysregulation of inflammatory responses plays a central role in the aetiology of many of these neonatal disorders. There is evidence to suggest that the omega-3 long chain polyunsaturated fatty acid docosahexaenoic acid (DHA) can down-regulate local and systemic inflammation in adults and animal models; however, very little is known about its protective effects in infants, especially preterm infants. Due to their immunological immaturity, preterm infants are particularly sensitive to diseases with an inflammatory aetiology in the early postnatal period. This makes DHA supplementation immediately after birth to combat neonatal inflammation an attractive therapy. Mechanistic data for DHA use in preterm infants are lacking and results from adult and animal studies may not be relevant to this population because of fundamental immune system differences. While there is increasing evidence from randomised controlled trials to support a beneficial effect of DHA for the preterm infant, more evidence is required to establish short and long-term effects of DHA on the immune status of preterm infants.

1. Introduction

Preterm birth, defined as birth at less than 37 completed weeks gestation, occurs in around 12% of deliveries worldwide with major implications for the long term health of the child [1,2]. Mortality rates of preterm infants have decreased substantially over the last few decades due to advancements in medical care [1]. However, morbidity rates, particularly in the very preterm infant (born less than 28 weeks gestation) have continued to rise [1]. Functionally and immunologically immature, the very preterm infant requires intensive support, and the medical interventions necessary for their survival can trigger a local or systemic inflammatory response [3].

Preterm infants have an under-developed immunoregulatory system, therefore there is the potential for chronic inflammation to develop [4]. Dysregulation of inflammatory responses plays a central role in the aetiology of many life-threatening neonatal disorders including bronchopulmonary dysplasia (BPD), necrotising enterocolitis (NEC) and sepsis [3–7] and presents a continuing challenge to clinicians involved in their care. Interest is intensifying in dietary compounds that promote resolution of inflammation and confer a protective effect against development of neonatal inflammatory disorders [8]. There is some controversy as to whether or not preterm infants can synthesise sufficient long-chain polyunsaturated fatty acids (LCPUFA) such as docosahexaenoic acid (DHA) and arachidonic acid (AA) from essential fatty acid (EFA) precursors [9–13] because genetic variants in the fatty acid desaturase genes may affect rates of synthesis of LCPUFA [13,14]. However, all infants receive an exogenous source of EFA and/or LCPUFA, via breast milk, lipid emulsions, formula or a combination of these sources [15]. Interest in DHA supplementation and its

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effect on clinical outcomes in preterm infants has escalated because these sources do not provide sufficient levels of DHA for these infants [16]. This review will focus specifically on the potential for the omega-3 LCPUFA DHA to act as an immunoregulatory agent to improve clinical outcomes in preterm infants.

2. Omega-3 LCPUFA and their role in early immune development

Preterm infants have a fundamentally different immune system to that of an adult or even a term infant, making them especially susceptible to an exaggerated immune activation [3]. The preterm infant relies heavily on the non-specific innate immune response for defence [3,17]. The antigen-specific adaptive immune system of preterm infants is also underdeveloped at birth, particularly with regard to T cells mediating inflammatory responses (T helper 1: Th1) and the important T cells involved in regulating the immune response (T-regulatory: T-reg) [3,18–20]. Ineffective T-reg function after birth, when the infant is exposed to a massive environmental antigenic onslaught from the birth process and neonatal intensive care unit (NICU), can result in excess inflammation and a lowered ability to down-regulate immune responses once initiated.

Breast milk has long been considered the gold standard for infant nutrition and is essential for promoting appropriate immune development in newborns [21]. In addition to a full complement of LCPUFA, breast milk also contains a complex mixture of immunologically active components such as growth factors, lactoferrin, prostaglandins, immunoglobulins, cytokines and immune cells [22,23]. Together with LCPUFA, the immunoregulatory bioactives in breast milk such as interleukin (IL) 10, transforming growth factor (TGF) β and DHA serve as mediators to promote oral tolerance and they also modulate developing immune responses while the infant develops their own immunoregulatory ability [3,19,20,24–28]. This immune maturation is crucial in order for a complex and dynamic relationship to develop between the innate and adaptive immune system [4,18,19,29], allowing infants to respond effectively and appropriately to self and pathogenic environmental stimuli [25,30]. Without appropriate regulation, an unchecked inflammatory pathophysiology can result, leading to many neonatal morbidities [3,4,17].

3. Inflammatory disorders in the neonate and the role of DHA

A heightened immune response leading to an exaggerated release of inflammatory mediators is a hallmark of BPD and other inflammatory disorders in the neonatal period, such as sepsis, NEC and retinopathy of prematurity (ROP) [3]. These disorders have a multi-factorial pathogenesis for which a single medication or comprehensive treatment is not available. Data from both preterm infant [4,31] and animal studies [32,33] support the potential for DHA to serve as a general preventative agent against inflammation without inhibiting development or function of underdeveloped organs.

3.1. Bronchopulmonary dysplasia

BPD is a disorder of prematurity characterised by the need for assisted ventilation or supplemental oxygen at 36 weeks post-menstrual age and signs of impaired alveolarisation and vasculogenesis in the lungs [4,34]. BPD occurs in approximately 45% of infants born less than 29 weeks gestation that survive preterm birth [4,35]. Ongoing lung damage may be caused by the preterm infant's inability to down-regulate and maintain control of the inflammatory immune response, leading to a chronic inflammatory state [35–38]. Decreased levels of DHA have been found to be associated with respiratory disease in preterm infants [39,40] and results from a prospective observational study in preterm infants supports this trend [41]. The best evidence for the ability of DHA to improve respiratory outcomes in preterm infants comes from our "*Docosahexaenoic acid for Improvement in Neurodevelopmental Outcomes (DINO) trial*". In the subgroup of infants born weighing less than 1250 g, those who received higher-DHA breast milk or formula had a reduced rate of BPD [42]. A recent meta-analysis supports the potential for DHA as a preventative agent against adverse respiratory outcomes when administered early in life [4]. These data support the concept that there is an early window of opportunity for effective immunomodulation with DHA; the critical period is when the immune system is still developing and before clinical phenotypes have been established in the infant [8,43].

3.2. Necrotising enterocolitis

NEC is predominantly a disease of prematurity, it is the most common gastrointestinal illness in newborns and has a high mortality rate [44–46]. As the disease progresses, inflammation in the intestine worsens causing breakdown of the mucosal barrier and an escalating immune cascade leading to sepsis, shock and even death [6,44,47]. The risk for developing NEC is strongly influenced by commensal bacteria, which exert metabolic, nutritional and immunological effects on the host [48]. A preterm infant has very low bacterial diversity and the establishment of a more complex microbiome is easily disrupted by events related to premature birth, for example, early antibiotic administration and Caesarean sections [49–51]. This process, termed dysbiosis, is implicated in the development of both sepsis and NEC [48].

Breast milk is the first choice for nutrition in the preterm infant and its early introduction is critical due to known gastrointestinal benefits [52]. Breast milk promotes bacterial colonisation of the gut, which in turn, is a major promoter of the development of immunoregulatory pathways required to mediate inflammation and bring about immunological homoeostasis [53]. Enteral feeding regimens for preterm infants consist of breast milk, preterm formula, or more commonly in the first few weeks, a combination of the two [52]. Both are sources of DHA which has been shown to influence the composition of the microbiome, albeit with controversial efficacy [32,54–56]. It has been proposed that fat intake and type of fat (saturated vs. unsaturated) influences the distribution of beneficial and protective bacteria in preterm infants [51]. A meta-analyse of trials in which NEC was reported has shown no benefit for omega-3 LCPUFA [4]; however none of the included trials were specifically designed nor powered to determine the true effect of DHA on NEC. Data from neonatal animal models is promising, as it has been reported that omega-3 LCPUFA-enriched diets support the colonisation of beneficial bacteria and protect against growth of pathogenic bacteria [32] and are protective against NEC [57]. Further, large-scale studies are required to first determine if DHA can reduce the incidence of NEC, and secondly if it is through a direct anti-inflammatory action or if DHA influences microbial communities directly in the gut.

3.3. Sepsis

Sepsis is a systemic inflammation caused by infection. Globally, sepsis is responsible for approximately 15% of neonatal deaths [58], with rates of infection dependent on the geographic region [5,59,60]. In preterm infants, sepsis is classified as either early-onset (<72 h of life) or late-onset (>72 h of life), with the latter being a common complication associated with prolonged admission to NICU [59,60]. The distinction between the two is of clinical importance, as early-onset sepsis usually results from exposure to

bacteria in utero or during delivery and late-onset sepsis is acquired from bacteria in the environment (ie. nosocomial infections) [59]. Different microorganisms are responsible for the pathogenesis of sepsis; bacterial infections are most common, but fungal, viral and parasitic infections are possible as well [59]. Decreased DHA has been associated with an increased risk of late-onset sepsis in preterm infants [39]. An appropriate balance of omega-3/6 LCPUFA has an impact on disease risk and alterations in these LCPUFA could be responsible for immune dysregulation and subsequent increase in sepsis risk [39].

3.4. Retinopathy of prematurity

ROP is the second leading cause of childhood blindness worldwide [61,62]. Impaired vasculogenesis and improper retinal neuronal development are responsible for the pathogenesis of ROP [63]. Because DHA is an integral part of cell membrane phospholipids, it may protect against the processes that impair vascular formation in ROP and thus may help to ameliorate vascular abnormalities and disease development [64–67]. Although a recent meta-analysis has shown no clear trend in risk for ROP between groups supplemented with omega-3 LCPUFA [4], a single study has reported that omega-3 LCPUFA supplementation was associated with a reduction in the incidence of ROP in preterm infants [65].

Results from clinical studies in preterm infants and animal studies supports the need for further large-scale randomised-controlled trials to determine the clinical efficacy of DHA supplementation to prevent inflammatory conditions in preterm infants.

4. Potential mechanisms for Omega-3 LCPUFA in the regulation of inflammation

The growing body of evidence suggests that dietary intake of LCPUFA early in life could influence immune development and other health outcomes. While there is extensive literature on the mechanisms of action of DHA and inflammation in adult disease, the targets that DHA acts on to exert its influence during initial immune development and resulting clinical conditions remains unclear. Studies in adults and animal models show that DHA influences a wide variety of mechanisms; some of which may be relevant to the neonate. These mechanisms include alterations in cell signalling pathways via changes to lipid rafts and cell membrane composition [68], modifications to receptor-mediated pathways such as PPARg, NFκB or GPR120 to inhibit or attenuate inflammation [69] [70] and increases in anti-inflammatory prostaglandin synthesis [71]. DHA may also influence the gut microbiome to promote immune regulation [54,56] and decrease oxidative stress [72]. However, there is a large gap in the literature relating to which aspects of inflammation are responsive to DHA in a neonate, or more specifically, in a preterm infant [73]. Preterm infants are an immunologically unique population and therefore mechanistic data pertaining to DHA's action in adults may not appropriate to predict efficacy in this population. In the following sections we review the evidence for the immunoregulatory efficacy of DHA, the effect of downstream metabolites of omega-3 LCPUFA and oxidative stress in preterm infants and preterm models (*in vitro* and animal studies). Important trends in data from studies utilising LCPUFA supplementation in term infants is also included when relevant or when data in preterm infants are unavailable.

4.1. DHA and the immune response: cytokine synthesis and release

In preterm infants, LCPUFA supplementation has been reported to modulate cytokine synthesis and immune cell phenotypes [74]. In a double-blind, randomised-controlled clinical trial in preterm infants, a medium-chain triglyceride/omega-3 LCPUFA emulsion was found to attenuate the inflammatory response compared to a soy (omega-6) emulsion [75]. Pro-inflammatory cytokines (IL-6 and IL-8) were significantly reduced at study-end in the omega-3 LCPUFA group [75], highlighting the potential for omega-3 LCPUFA to dampen the heightened immune response seen in preterm infants after birth. The addition of DHA and AA (omega-6 LCPUFA) to standard infant formula has been shown to increase the production of immunoregulatory IL-10, reduce IL-2 and increase the proportion of antigen-mature memory ($CD45RO^+$) $CD4^+$ cells (important in adaptive immunity) in infants to levels comparable to those seen in the breast milk-fed group [74], indicating an effect of DHA on immune maturation [74]. Furthermore, Gold et al. (2006) observed that omega-3 eicosapentaenoic acid (EPA) and AA reduced antigen- and mitogen-stimulated IFNγ production *in vitro* in cord blood samples from a US birth cohort study (30–42 weeks gestation) [76]. These data support the potential for omega-3 LCPUFA to inhibit release of pro-inflammatory cytokines by Th1 cells. Additionally, lymphocyte proliferation was reduced by EPA and AA, indicating an attenuation of the immune response by both omega-3 and 6 LCPUFA [76].

While immune response data from preterm infants are limited, data from term infants are supportive of an immunoregulatory role for DHA. Cellular immune responses were compared in term neonates who received either standard formula, formula supplemented with DHA/AA or breast milk from 2 to 6 weeks of life [77]. Although results were not significantly different, the infants that received the formula supplemented with DHA/AA had similar cytokine and T cell responses to those in the breast milk group [77]. Cultured lymphocytes from both formula groups produced more pro-inflammatory cytokines than the human milk fed group. However at 6 weeks, an increased pro-inflammatory TNFα response was seen in the formula group but not for the formula + DHA/AA group [77]. In addition, the group without LCPUFA supplementation exhibited less maturation of the adaptive immune response (fewer CD4+CD28 + cells) and they had significantly fewer peripheral blood effector memory T cells (CD3+CD44 + cells), which suggests that these fatty acids influence the degree of T cell maturation and development of effector memory T cells [77] [78]. A decrease in pro-inflammatory cytokines in response to *in vitro* stimulation by allergens (ovalbumin, house dust mite, cat hair, phytohaemagglutinin) was also reported for immune cells from term infants, whose mothers were supplemented with fish oil (3.7 g/day omega-3 LCPUFA) during pregnancy [79]. The data supports a role for early dietary LCPUFA in immune development and regulation.

Animal studies also reinforce the anti-inflammatory role of omega-3 LCPUFA seen in preterm and term infants. A reduction in systemic inflammation would be reflected by a reduction in pro-inflammatory mediators and/or the increase in regulatory cytokines. In a neonatal piglet model, increased dietary DHA and decreased omega-6 LCPUFA was associated with a reduction in systemic inflammation (as measured by C-reactive protein (CRP)) [33]. Mice receiving an omega-3 LCPUFA supplemented diet produced offspring with significantly increased immunoregulatory IL-10 levels in the colon and spleen [54]. Importantly, variations in the local tissue cytokine milieu also have the potential to influence other immune cells, such as antigen presenting cells (APC) that are critical for initiating immune responses [80]. DHA-induced changes in the cytokine environment may have downstream effects on priming of APC and other immune cells for the promotion of an anti-inflammatory response during initial antigen encounter [8,80].

4.2. Role of DHA in the resolution of inflammation: resolvins

Downstream metabolites of LCPUFA, such as resolvins and protectins, are also important in mediating an anti-inflammatory response. Resolvins are directly synthesised metabolites of omega-3 LCPUFA and play a key role in terminating inflammatory responses [81]. These metabolites promote resolution and tissue repair and importantly for the preterm infant this occurs without compromising host defence [81]. Much of the work that examines the interaction between LCPUFA, resolvins and inflammation has been conducted *in vitro* in samples obtained from adult and animal models, leaving a large gap in knowledge for the effectiveness in neonates. Results from neonatal animal models serve as the best indicator for effectiveness of LCPUFA's ability to trigger resolution of inflammation. Martin et al. (2014) recently examined the effect of supplementation with a downstream metabolite of DHA, Resolvin D1 (RvD1), along with a downstream metabolite of AA, lipoxin A4 (LxA4), in a neonatal mouse model of BPD [82]. The results demonstrate that these metabolites reversed the histologic and biochemical changes associated with lung injury in the mouse model. Whilst RvD1 and LxA4 were shown to reduce alveolar damage when administered on their own, the most significant reduction in alveolar damage was seen when the two were combined [82]. Similarly, Velten et al. (2014) also reported that prenatal DHA supplementation reduced neonatal lung inflammation in a mouse model [83]. These recent findings suggest biological plausibility for a mechanistic role of terminal metabolites of DHA in combatting inflammatory lung damage.

4.3. DHA and the mediation of oxidative stress

Supplemental oxygen therapy, while vital to the survival of infants with BPD, also places a large oxidative stress burden on the infant [7]. Furthermore, if the infant is also receiving parenteral nutrition, the lipids are subjected to an increased risk for peroxidation while exposed to supplemental oxygen. DHA and its downstream metabolites have been reported to reduce oxidative stress [84]. A double-blind randomised controlled trial in preterm infants (n = 38) reports that a parenteral lipid emulsion containing omega-3 LCPUFA reduced oxidative stress compared to standard lipids (soy) [85]. Oxidative stress levels were determined by Vitamin A, E and total antioxidant potential. Positive results with respect to omega-3 LCPUFA and oxidative stress were also obtained when very preterm infants (n = 30) were randomised to either a lipid emulsion containing soy and olive oils (ClinOleic®) or one containing soy, medium-chain triglycerides, olive oil and fish oil (SMOFlipid®) [86]. Vitamin E levels and plasma F2-isoprostanes (lipid peroxidation marker) were used to quantify levels of oxidative stress in the infants. Compared to ClinOleic®, SMOFlipid® reduced oxidative stress in this high risk infant population and was determined to be safe and well-tolerated [86].

Results from research conducted in children and adolescents with acute lymphoblastic lymphoma (ALL) are relevant as they add strength to the use of DHA as an adjuvant therapy to combat oxidative stress in preterm infants. In a randomised, double-blind, placebo-controlled trial, participants (n = 70) received either 1000 mg/day fish or placebo oil in combination with their methotrexate regimen [87]. Methotrexate is a necessary treatment in patients with ALL but is known to induce hepatotoxicity, the pathogenicity of which is mainly due to oxidative stress [87]. Omega-3 LCPUFA as an adjunct therapy in these patients ameliorated the hepatotoxicity compared to placebo and no adverse reactions to the fish oil were observed [87]. These results are promising; however, more in-depth studies are required in larger cohorts to establish the robustness of these findings and the mechanisms underlying the action of DHA against oxidative stress.

In a neonatal guinea pig model indicators of oxidative stress (peroxide and aldehyde concentrations) were reduced when guinea pigs were fed an omega-3 LCPUFA emulsion compared to an omega-6 LCPUFA emulsion [88]. Levels of pro-inflammatory IL-1 and tumour necrosis factor alpha (TNFα) mRNA were also significantly reduced in the fish oil emulsion group [88]. Further research in humans to determine appropriate dose and timing regimens is required to reveal whether DHA would serve as an effective adjunct therapy to combat oxidative stress in infants the NICU.

5. Implications and conclusions

There is increasing experimental evidence and a convincing rationale for DHA supplementation to improve clinical outcome in preterm infants by targeting the critical window of time where immunomodulation has the greatest potential for benefit [4,89]. However, several areas require further consideration and research. First, further large-scale interventions are required in order to determine the clinical efficacy of DHA during the neonatal period in this unique and vulnerable population. Second, to optimise DHA's benefit on clinical outcomes, it is essential that the sites of action of both the fatty acid and its downstream mediators on the preterm infant's immune system are thoroughly elucidated. This knowledge would allow clinicians to determine whether DHA would be best used as a preventative versus treatment option in preterm infants. Lastly, the mechanisms of action of DHA on the immune system in the short and long-term must be examined.

It is anticipated that the results from a large-scale randomised clinical trial of enteral DHA supplementation on respiratory outcomes and other parameters in preterm infants that is currently underway in Australia, New Zealand and Singapore will answer some of these questions and guide future research and recommendations. The N3RO trial (n-3 LCPUFA for the improvement in Respiratory Outcomes- ACTRN trial #12612000503820) will shed light on the mechanisms of action of omega-3 LCPUFA on the immune system while it is still functionally immature.

Author contributions

All authors contributed to conceptualisation and critical review of the literature. All authors were involved in the writing, editing and final review of the manuscript. All authors have read and approved the final version of the manuscript.

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The Women's and Children's Health Research Institute has a patent “Methods and compositions for promoting the neurological development of an infant” on which MM and RAG are listed as inventors. The patent has been accepted in Australia and is pending in the USA, Canada and Malaysia.

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