Association of Fish Oil Containing Lipid Emulsions with Retinopathy of Prematurity: A Retrospective Observational Study

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Abstract

Background: Retinopathy of prematurity (ROP), commonly occurred in preterm infants, remains a leading cause of childhood blindness worldwide. Preterm infants have less body storage of docosahexaenoic acid (DHA), which is an important component of the retina and optic nerve. Therefore, this study aimed to investigate whether supplementation of n-3 polyunsaturated fatty acids (n-3 PUFAs) in parenteral nutrition may have beneficial effects on ROP in preterm infants.

Methods: A total of 89 preterm infants, admitted to Neonatal Intensive Care Unit (NICU) in Anhui Provincial Children's Hospital from September 2017 to August 2020, were recruited in the study. Based on the medical documents, the subjects were categorised into two groups: the fish oil emulsion group (n=43) in which infants was given parenteral Multi-oil emulsions, containing soy oil, medium-chain-triglyceride, olive oil and fish oil (6g/dL, 6g/dL, 5g/dL and 3g/dL respectively), and the soy oil emulsion group (n=46) in which infants was received intravenous LCT-MCT emulsions, containing 10g/dL of soy oil and medium-chain triglyceride each. ROP was screened and diagnosed in terms of the location (three zones) and abnormal vascular response at the junction of the vascularized and avascular retina (five stages) of retinopathy, and plus disease. Fatty acids in erythrocytes were determined using gas chromatography.

Results: After 4 weeks of hospitalization, among all the preterm infants, 39 developed ROP (all stages) indicating an incidence of ROP at 43.82%. The incidence of ROP showed no differences between the two groups, irrespective of the stage of ROP. However, the incidence of severe ROP in the group with fish oil emulsions was significantly lower than that in the group with soy oil emulsions ($P<0.05$). After 14 days of nutrition support, the preterm infants administered fish oil emulsions had an increase in erythrocyte DHA content, with a reduction in the ratio of arachidonic acid (AA) to DHA and an increase of n-3 index.

Conclusion: Supplementation of n-3 PUFAs through parenteral fish oil containing lipid emulsions resulted in an increase in body DHA quantity, and this might have beneficial effects on prevention of severe ROP in preterm infants.

Background

Retinopathy of prematurity (ROP) is a potentially sight-threatening neurovascular disease which affects retina in infants, especially in preterm infants [1, 2]. In 1943, two epidemic episodes of vision impairment and blindness in infants have been reported in high-income countries; then, in the mid-90s, the third one occurred in Latin America and Europe, which were all due to ROP [3]. In the 21st century, the accomplishment of neonatal health care system supports a higher survival rate of preterm infants than ever before, leading to an increased incidence of ROP worldwide. Although there has been considerable improvement in neonatal care and in our understanding and treatment of ROP, ROP remains a leading cause of childhood blindness worldwide [4]. It has been demonstrated that prematurity and long-term high oxygen supplementation are the major risk factors; whereas, maternal diseases (hypertension, diabetes mellitus, etc.), caesarean section, premature rupture of membranes, respiratory distress

syndrome (RDS), low Apgar score, patent ductus arteriosus (PDA), necrotising enterocolitis (NEC), intraventricular haemorrhage (IVH) and sepsis are considered as alternative risk factors [4, 5].

It is clear that docosahexaenoic acid (DHA) (20:6n-3), belonging to the series of n-3 PUFAs, is an important component of retinal cell membrane and is required for photoreceptor cell development [6, 7]. Highest body concentrations of DHA per unit area are found in the disc membranes and the overall percentage of DHA (30% of total retinal fatty acids) is 50 mol% greater than in the next most concentrated tissue [8]. Compared with full-term infants, preterm infants have lower plasma level and less body storage of DHA, because they have missed important intrauterine accretion of nutrients including DHA in the third trimester. Furthermore, preterm infants are with poorer capability to synthesize DHA from its precursor α-linolenic acid (ALA) (18:3n-3) [9]. Consistent evidence demonstrates that n-3 PUFAs may act in a protective role against ischemia-, light-, oxygen-, inflammatory-, and age-associated pathogenesis of vasoproliferative and neurodegenerative retinal diseases such as ROP [8, 10].

Nutrition support including enteral and parenteral nutrition is essential for preterm infants, satisfying the daily energy and nutrient requirements for appropriate growth and development, as well as disease treatment and prevention [11]. Lipids are important components of parenteral nutrition to meet the requirements for essential fatty acids and high energy in preterm infants, who have limited endogenous lipid storage. Lipid emulsions are available for use as part of parenteral nutrition, including conventional lipid emulsions consisting of pure soybean oil, mixed lipid emulsions consisting of soybean oil plus medium-chain triglycerides (MCTs) and/or olive oil, and most recently, a multicomponent lipid emulsion comprising 30% soybean oil, 30% MCTs, 25% olive oil, and 15% fish oil [12]. Soybean oil-based lipid emulsions containing primarily n-6 polyunsaturated fatty acids (n-6 PUFAs), have been demonstrated to contribute to increased body levels of proinflammatory cytokines and oxidative stress. Multicomponent lipid emulsions comprising fish oil n-3 PUFAs, growingly accepted during the past decades, may be beneficial for paediatric patients by supplying a balanced ratio of n-6 to n-3 PUFAs [13, 14], but more evidence is needed particularly for preterm infants, regarding changes in blood fatty acid levels and potential health risk without strong evidence of benefit [15–17]. Therefore, in this retrospective observational study, we investigated the effects of fish oil n-3 PUFA containing lipid emulsions on body accumulation of DHA, and their associations with ROP in preterm infants.

Methods

Subjects

Based on the estimated sample size (from 19 to 192) with $\alpha = 0.05$ and $\beta = 0.1$, and occurrence rate of ROP in preterm infants, a total of 89 preterm infants, admitted to Neonatal Intensive Care Unit (NICU) in Anhui Provincial Children's Hospital from September 2017 to August 2020, were recruited in the study. Inclusion criteria were as follows: BW < 2000 g and GA < 34 weeks, and supported with parenteral nutrition longer than 14 days. Exclusion criteria were as follows: being with one or more major congenital malformation, inborn metabolic disorders, and having signs or symptoms of congenital infection. The

detailed information was recorded on the preterm infants' gestational age (GA), delivery mode, premature rupture of membranes, birth weight (BW), gender, Apgar score, duration of oxygen use, biochemical parameters, accompanied diseases (PDA, RDS, NEC, IVH, sepsis), and maternal diseases (hypertension, diabetes mellitus). Heparin-anticoagulant peripheral blood samples, which were left after used by diagnosis purpose and stored at -80°C, were collected for analysing fatty acids in red blood cells. The institutional Review Board/Ethics Committee of Anhui Provincial Children's Hospital reviewed and approved this retrospective, observational study, and the legal guardians gave written consent for their participation.

Based on the medical documents, the subjects were categorised into two groups: fish oil emulsion group (n = 43) in which infants was given parenteral Multi-oil emulsions, containing soy oil, medium-chain-triglyceride, olive oil and fish oil at concentrations of 6g/dL, 6g/dL, 5g/dL and 3g/dL respectively (Fresenius Kabi Austria GmbH); and soy oil emulsion group (n = 46) in which infants was received intravenous LCT-MCT emulsions, containing 10g/dL of soy oil and medium-chain triglyceride each (Fresenius Kabi China GmbH). The initial emulsion dose was 0.5 to 1.0 g of lipids per kg of body weight, and was increased by 0.5 to 1.0 g of lipids per kg body weight every 24 hours with a maximum of 3.0 to 3.5 g of lipids per kg of body weight/d.

Ophthalmologic Assessment

For all the preterm infants, ROP was screened and diagnosed in terms of the location (three zones) and abnormal vascular response at the junction of the vascularized and avascular retina (five stages) of retinopathy, and plus disease, according to the International Classification of Retinopathy of Prematurity. Severe ROP was defined as the level of stage 3 or higher and plus disease, with a higher risk of an unwanted outcome [18, 19]. The screening examination, conducted by one ophthalmologist trained in the diagnosis of ROP, started at 4 weeks of postnatal age, and the follow-up examination was conducted at 6 weeks of postnatal age.

Fatty acid analysis in erythrocytes

Fatty acids in erythrocytes were determined using gas chromatography. Fatty acid methyl esters from red blood cells were prepared according to a modified method of Lepage [20, 21]. The same procedure was followed as our previous described [22] on an Agilent 6890 N GC system, and the quantity of fatty acids was expressed as the percent (%) (wt/wt) of the total fatty acids.

Statistical Analysis

Continuous data were expressed as means ± SD, and categorical data were expressed as percent (%). The t-test, Chi-square test, Mann-Whitney U-test or Fisher's exact test was used as appropriate. Statistical analysis was performed on an Apple MacBook laptop by using Statistical Product and Service Solutions (SPSS) software, version 26. $P < 0.05$ was considered as significant difference.

Results

General information for the preterm infants

The characteristic of subjects in this study was presented in Table 1. The preterm infants administered fish oil emulsions had a lower rate of caesarean section, compared to that with soy oil emulsions ($P < 0.05$). No differences were found between the two groups of infants with regard to other parameters including BW, GA, gender, premature rupture of membranes, Apgar score, duration of oxygen use, biochemical parameters, accompanied diseases (PDA, RDS, NEC, IVH, sepsis), and maternal diseases (hypertension, diabetes mellitus).

Table 1
Clinical characteristics of preterm infants with soy oil emulsions and fish oil emulsions

Variables	Group with soy oil emulsions (n = 46)	Group with fish oil emulsions (n = 43)	P value
General information	1594 ± 296	1596 ± 263	0.674
Birth weight (g)			
Gestational age (wk)	31.9 ± 2.3	31.6 ± 2.3	0.543
Male gender, n (%)	26 (56.5)	27 (62.8)	0.547
Premature of rupture membranes (h)	27.91 (0-384)	41.88 (0-1440)	0.395
Caesarean section, n (%)	28 (60.9)	17 (39.5)	0.044
Accompanied diseases			
PDA, n (%)	4 (8.7)	7 (16.3)	0.277
NEC, n (%)	6 (13)	1 (2.3)	0.112
IVH, n (%)	29 (63)	27 (62.8)	0.980
Sepsis, n (%)	1 (2.2)	1 (2.3)	1.000
RDS, n (%)	26 (56.5)	30 (69.8)	0.197
Plasma biochemical parameters *			
Glucose (mmol/L)	3.05 ± 1.89	2.27 ± 1.58	0.570
Creatinine (μmol/L)	56.51 ± 25.49	53.83 ± 16.79	0.967
Alanine aminotransferase (μmol/L)	10.70 ± 28.25	4.97 ± 4.30	0.153
Bilirubin (μmol/L)	50.64 ± 28.83	46.92 ± 13.92	0.965
Albumin (g/L)	33.12 ± 3.49	33.74 ± 2.75	0.371
Maternal diseases			
Diabetes mellitus, n (%)	1 (2.2)	2 (4.7)	0.608
Hypertension, n (%)	10 (21.7)	7 (16.3)	0.513

* Examined within 24 h of admission in the first 48 h after birth and before parenteral nutrition started. PDA, patent ductus arteriosus; NEC, necrotising enterocolitis; IVH, intraventricular haemorrhage; RDS, respiratory distress syndrome.

ROP incidence in the preterm infants

After 4 weeks of hospitalisation, among all the preterm infants, 39 developed ROP (all stages) indicating an incidence of ROP at 43.82%. As shown in Table 2, the incidence of ROP showed no differences between the two groups, irrespective of the stage of ROP of 46 infants in the group with soy oil emulsions, 16 developed ROP, whereas of 43 infants, 23 developed ROP in the group with fish oil emulsions. However, the incidence of severe ROP in the group with fish oil emulsions was significantly lower than that in the group with soy oil emulsions ($P < 0.05$). At 6 weeks of postnatal age, the incidences of any stage and severe ROP were similar to them at 4 weeks of postnatal age, which only had one more stage I case in soy oil emulsion group.

Table 2
Changes in erythrocyte fatty acids with administration of lipid emulsions in preterm infants

	Before PN		After 14 d of PN	
Variables	Group with soy oil emulsions (n = 46)	Group with fish oil emulsions (n = 43)	Group with soy oil emulsions (n = 46)	Group with fish oil emulsions (n = 43)
Total SFAs	55.98 ± 5.87	55.10 ± 6.32	50.98 ± 2.82 *	53.30 ± 6.05
Total MUFAs	16.92 ± 2.50	17.20 ± 2.04	18.14 ± 2.44	18.31 ± 2.38
Total n-6 PUFAs	20.10 ± 5.38	21.16 ± 5.24	24.45 ± 2.77 *	21.23 ± 5.01
C18:2n-6	5.01 ± 2.74	5.85 ± 2.91	9.70 ± 2.74 *	7.40 ± 3.36
C18:3n-6	0.21 ± 0.06	0.20 ± 0.05	0.19 ± 0.05	0.19 ± 0.06
C20:4n-6	14.49 ± 4.73	14.60 ± 4.79	14.35 ± 2.97	13.23 ± 4.30
C22:5n-6	0.39 ± 0.20	0.51 ± 0.18	0.21 ± 0.17	0.41 ± 0.20
Total n-3 PUFAs	7.05 ± 1.28	6.58 ± 1.32	6.49 ± 1.45	7.18 ± 1.87
C18:3n-3	2.80 ± 0.38	2.55 ± 0.42	2.51 ± 0.79	2.38 ± 0.80
C20:5n-3	2.42 ± 0.62	2.33 ± 0.71	2.47 ± 0.65	2.42 ± 0.77
C22:6n-3	1.83 ± 0.73	1.71 ± 0.48	1.52 ± 0.64	2.37 ± 0.55 *
n-6/n-3 PUFAs	2.85 ± 0.69	3.21 ± 0.59	3.77 ± 1.24	2.96 ± 1.73
AA/DHA	7.93 ± 4.71	8.54 ± 2.38	9.46 ± 2.53	5.57 ± 2.18

PN, parenteral nutrition. * Compared to the same lipid emulsions before PN administration, $P < 0.05$.

Changes in erythrocyte fatty acids in the preterm infants

The fatty acid compositions in erythrocytes from the two groups of preterm infants with soy oil and fish oil emulsions were shown in Table 3. Before parenteral nutrition performed, no differences in each fatty acid composition were found between the two groups. However, after 14 days of nutrition support, the preterm infants administered fish oil emulsions had an increase in DHA content in erythrocytes, with a reduced ratio of arachidonic acid (AA) (20:4n-6) to DHA. Whereas, those given soy oil-based emulsions had an increase in content of linoleic acid (LA) (18:3n-6) and total n-6 PUFAs, concomitant with a reduction of total saturated fatty acids.

Table 3. Differences in ROP incidence in preterm infants between Soy oil emulsion and fish oil emulsion administration

Variables	Group with soy oil emulsions (n=46)		Group with fish oil emulsions (n=43)	
	4 wk	6 wk	4 wk	6 wk
ROP (all stages), n (%)	16 (34.78)	17□36.95□	23 (53.48)	23 (53.48)
stage I	8	9	11	11
stage II	8	8	12	12
Severe ROP, n (%)	11 (23.91)	11 (23.91)	1 (2.33)*	1 (2.33)*

* Compared to the soy oil emulsions at the same time point, $P<0.05$.

Discussion

It has been increasingly recognized that ROP differs worldwide and tailored screening and treatment approaches are needed to reduce aberrant vasoproliferation and facilitate physiologic retinal vascular development in infants [4]. N-3 PUFAs are essential for normal retinal development and appear to play a protective role against retinal neovascularization and visual damage, if provided with n-3 PUFA-enriched formulas in preterm neonates [10]. DHA and its substrate eicosapentaenoic acid (EPA) (20:5n-3), being regulators for transcription factor, and parent fatty acids for neuroprotectin D1 and a family of eicosanoids, have beneficial effects on ischemia, oxidative stress, inflammation and cellular signaling mechanisms, influencing retinal cell gene expression and cellular differentiation, as well as activating molecules implicated in the pathogenesis of vasoproliferative and neurodegenerative retinal diseases such as ROP [10]. In our study, we found that intravenous fish oil containing lipid emulsions increased erythrocyte DHA content with no changes in EPA content and reduced the incidence of severe ROP in preterm infants, compared to the traditional soy oil lipid emulsions.

Concerning the effects of n-3 PUFA supplementation on ROP in preterm infants, no final conclusions have yet been reached. In a prospective cohort study, it is found that a time-dependent accumulation of AA at the expense of DHA seems to occur in utero in erythrocytes of preterm infants who will develop ROP, thus reinforcing the beneficial properties of DHA on this disease [23]. In other observational or prospective

randomized studies, the use of intravenous fish oil-containing lipid emulsions is associated with a lower incidence of ROP or severe ROP and decreased need for laser or bevacizumab treatment in preterm infants [24–26], which is in keeping with our findings. Inconsistently, preterm infants treated with fish oil containing emulsions did not differ for any or severe ROP, as well as for bronchopulmonary dysplasia, NEC, PDA, or sepsis, and the incidence of cholestasis in extremely premature infants [27, 28]. Therefore, evidence is insufficient to determine with any certainty if fish oil emulsions offer advantage in prevention or resolution of ROP or in any other clinical outcome, and a large multicenter randomized clinical trial is required [29, 30]. Inconsistencies between studies may be ascribed to baseline differences in body n-3 PUFAs, different dosages used and duration, other nutrient deficiencies and lack of investigating interaction effects of gender and age, etc. [31].

The accretion of PUFAs including n-3 series as well as n-6 series in the fetus increases exponentially from the 30th to the 38th week during pregnancy, and continues during the first 3 years after birth, and thus preterm infants may be in more disadvantage compared to term infants if n-3 PUFAs provided insufficiently after birth [32]. It has been reported that preterm infants fed milk with a DHA content 2–3 times higher than the current concentration in infant formulas have better neurologic outcomes in early life, and thus suggesting that human milk and preterm formula should contain approximately 1.5% of fatty acid as DHA to compensate for the early DHA deficiency [33, 34]. Herein, our results showed that the erythrocyte DHA content was increased with parenteral administration of the fish oil containing emulsions to preterm infants, instead of the soy oil lipid emulsions, which caused an increase in LA and total n-6 PUFA content in erythrocytes. Similarly, providing a target dose of 3 to 3.5 g/kg/day of lipid emulsions containing 15% of fish oil beneficially modulates the DHA profile, but providing lipid emulsions containing 10% fish oil at a dose of $\leq$ 2 g/kg/day fails to increase circulating DHA [35].

In addition to DHA, EPA is found to be beneficial for body health by antagonizing arachidonic acid (AA) derived eicosanoids, which is associated with retinal neurovascular impairment [8]. The n-3 index, the percentage of EPA plus DHA in erythrocytes, represents a human body's status in EPA and DHA. The compiling recent data supports the target range for the omega-3 index of 8–11% in adults and pregnant mothers [36]. In children and adolescents, daily supplementation of $\geq$ 450 mg DHA + EPA and an increase in the n-3 index to > 6% makes it more likely to show efficacy on cognitive development [37]. In the current study, the n-3 index was elevated to 4.79% by the use of fish oil containing lipid emulsions with no changes in erythrocyte EPA content, but it was still less than the idea value (6%) for children. This might be resulted from the daily supplementation of less DHA + EPA (< 320 mg per day) in the lipid emulsions used. In consistency, other reports demonstrated that extremely premature infants on the same fish oil containing lipid emulsions with ours had significantly elevated fraction of EPA and slightly increased DHA fraction with the n-3 index of 4.03% at 14 days [27]. Next, supplementation of higher quantities of DHA + EPA using new lipid emulsion formulas and the optimal n-3 index need to be focused to elucidate their effects on the growth and development of infants including preterm babies.

To be noteworthy, evaluating postnatal AA status after birth and correcting its deficiency in addition to EPA and DHA need to be strengthened for ROP prediction and prevention, because AA is indispensable in

the vasculature and in specific aspects of immunity, being functioned as a precursor for leukotrienes, prostaglandins, and thromboxanes, collectively known as eicosanoids in infant development [38]. In term infant formula, ratios of n-6 to n-3 PUFAs around 7:1 have been most commonly used, and preterm formulas usually have the ratios ranging between 5:1 and 15:1 [39]. Several expert groups recommend that infants receive at least 0.3% DHA and at least 0.3% AA, with a ratio of AA to DHA from 1:1 to 2:1 in infant feedings, is associated with improved visual and cognitive outcomes [40]. The low postnatal AA levels has been proved to be strongly associated with the development of ROP (any stage of ROP and severe ROP) [41]. Furthermore, a randomized clinical trial study found that, compared with standard of care, enteral simultaneous supplementation of AA and DHA lowered the risk of severe ROP by 50% and showed overall higher serum levels of both AA and DHA [42]. In this study, erythrocyte AA content was not altered by parenteral administration of either soy oil or fish oil lipid emulsions, because body AA content is usually stable owing to the higher ability for AA synthesis from its precursor LA [39]. Nonetheless, the best clinical approach to PUFA supplementation and n-6 to n-3 PUFA (or AA to DHA) ratios are still far from evident, and requires in-depth investigation on specific fatty acid supplementation in the context of other fatty acids [39].

In conclusion, administration of fish oil containing lipid emulsions increased erythrocyte DHA content with an increased AA/DHA ratio and reduced n-3 index, even though EPA and AA content in erythrocytes was not changed, and consequently had beneficial effects on severe ROP in preterm infants. In order to provide a more conclusive picture, future trials should employ larger sample sizes in prospective randomized studies with long-term follow-up and should focus on supplementation of higher quantities of DHA + EPA.

Declarations

Ethics approval and consent to participate

The institutional Review Board/Ethics Committee of Anhui Provincial Children's Hospital approved the study (No. 2017ek008). The legal guardians gave written consent for their participation.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Dr Rongqiang Yang and Dr Kemin Qi conceptualised and designed the study, screened charts, extracted the data, analysed and interpreted the data, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr Hao Ding, Dr Jing Shan and Dr Xiaole Li conceptualised and designed the study, analysed and interpreted the data, and critically reviewed and revised the manuscript for important intellectual content.

Jian Zhang, Guanghui Liu, and Hong Zheng conceptualised and designed the study, critically reviewed and revised the manuscript for important intellectual content.

Dr Yu Su conceptualised and designed the study, analysed and interpreted the data, and critically reviewed and revised the manuscript for important intellectual content.

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