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The metabolic effects of two different lipid emulsions used in parenterally fed premature infants — A randomized comparative study

Gamze Demirel *, Serife Suna Oguz, Istemi Han Celik, Omer Erdeve, Nurdan Uras, Ugur Dilmen

Division of Neonatology Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey

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ABSTRACT

Objective: To compare the effects of two different lipid emulsions, based on soybean oil and olive oil respectively on plasma lipid concentrations and acylcarnitine profile of very low birth weight infants.
Design: Randomized comparative study.
Patients and methods: Forty very low birth weight infants, ≤32 weeks of gestational age and receiving at least 40% of the calorie taken by parenteral nutrition from lipid solution at 14th day of life were evaluated. Group I (n = 20) received soybean oil based lipid emulsion (Intralipid®) and Group II (n = 20) received olive oil based lipid emulsion (Clinoleic®).
Main outcome measures: Plasma lipid concentrations and acylcarnitine profile were assessed.
Results: Triglyceride, cholesterol, high and low density lipoprotein levels, liver function tests were similar between two groups whereas very low density lipoprotein level was statistically lower in Group I ($p<0.05$). Free carnitine levels were 15.73 ± 10.67 in Group I and 34.25 ± 22.18 μM in Group II ($p = 0.012$) and hexanoyl carnitine levels 2.18 ± 2.10 in Group I and 0.38 ± 0.12 μM in Group II, respectively ($p = 0.005$). Plasma medium chain acylcarnitine levels were significantly higher in Group I.
Conclusions: Low levels of very low density lipoprotein in Group I may be a way of hemostasis to keep the serum triglyceride within normal levels. Lower free carnitine levels in soybean oil-based group is the result of carnitine need during the mitochondrial transport of long chain fatty acids. In Group I, due to the inefficient transport of medium chain fatty acids into the mitochondria, medium chain acylcarnitines accumulate in plasma. This may be the reason of lower carnitine levels in Group I. We suggest that higher levels of hexanoyl carnitine, reflecting defective mitochondrial transport of hexanoyl which leads immunsupression, may be the cause of higher sepsis risk in Group I.

1. Introduction

Intravenous lipid emulsions are complex pharmaceutical products used for parenteral nutritional support. They are developed to supply calorie and essential fatty acids for patients who are unable to intake adequate nutrition enterally [1].

The exact composition of lipid solutions vary by different manifacturers. Widely used lipid emulsion based on soybean oil contains also egg yolk phospholipids and glycerol and contains long chain triacylglycerols (LCTs), 62% of which are polyunsaturated fatty acids (PUFAs), mainly linoleic acid (18:2n-6) [2]. Exposure to large amounts of PUFAs is associated with an increased risk of membrane peroxidation that leads oxidative stress in critically ill neonates. High levels of PUFAs also induces decrease in immune cell function [3]. The olive oil based lipid emulsion contains olive oil and soybean oil in a ratio of 4:1, containing high levels of monounsaturated oleic acid and α-tocopherol compared to soybean based formulations [4, 5]. It contains only LCTs, has a lower proportion (20%) of PUFAs and provides 60% monounsaturated fatty acids (MUFAs). Decreased levels of immunsupressive n-6 polyunsaturated fatty acid and increased content of α-tocopherol, a natural antioxidant, offer advantages for very low birth weight infants (VLBW) who are at risk for infections and oxidative injury [1]. There are studies that showed n-9 oleic acid or olive oil based on n-9 fatty acid did not inhibit lymphocyte proliferation and did not reduce the expression of activation markers on granulocytes [6–10]. The olive oil based lipid formula was evaluated in some trials in preterm infants and all of them showed good tolerability and no signs of essential fatty acid deficiency [11–17].

Beta-oxidation is the major degradative pathway for fatty acid esters in humans. The main function of carnitine is to serve as a carrier substance, transporting LCFA from the cytoplasm across the inner mitochondrial membrane into the mitochondrium. The formation of acylcarnitine and its efflux from the mitochondria provides a sink when acyl-Co A formation exceeds the rate of CoA recycling inside the mitochondria. Carnitine may also facilitate the transport of these

* Corresponding author at: Zekai Tahir Burak Maternity Teaching Hospital, Division of Neonatology, Ankara, Turkey. Tel.: +90 3123065270.
E-mail address: kgamze@hotmail.com (G. Demirel).

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excess acyl groups from the liver to other tissues for utilization [18]. Plasma acylcarnitine profile is a reflection of the mitochondrial acyl-CoA status. Analysis of plasma acylcarnitine levels can show many metabolic disorders of fatty acid and branched-chain amino acid. Although the effects of lipid solutions on plasma free fatty acid levels and lipid metabolism are well known, there are no data in literature about their effect on acylcarnitine profile of preterm infants whose metabolism is quite different from adults.

In this study, we aimed to compare the effects of two lipid emulsions, based on soybean oil (Intralipid®) and olive oil (Clinoleic®), on plasma lipid and acylcarnitine profile in preterm infants.

2. Patients and methods

This randomized prospective study was performed on VLBW (<1500 g) neonates who were followed from January 2010 to October 2010 in Zekai Tahir Burak Maternity Teaching Hospital Neonatal Intensive Care Unit. The patients whose gestational age was ≤32 weeks and receiving at least 40% of the calorie taken by parenteral nutrition (PN) from lipid solution at 14th day of life were included into the study. The patients were randomized in two groups according to the type of lipid emulsions. Using computer-generated randomisation, the lipids were assigned to the infants randomly. Group I received a soybean based lipid emulsion (Intralipid®, 20%, Fresenius Kabi AB, Uppsala, Sweden) and Group II received olive oil based lipid emulsion (Clinoleic®, 20%, Baxter/Eczacıbaşı, Istanbul, Turkey). The patients who had any metabolic disorders, congenital anomalies, severe unconjugated hyperbilirubinemia, infants using medications in competition with bilirubin, infants with birth asphyxia and who died within the first 14 day of life were excluded from the study. The study was approved by the local research ethics committee.

Parenteral nutrition solutions were prepared daily in our hospital pharmacy, PN unit, under a laminar flow by Exacta-Mix™ compounder system (Baxa Corporation, USA) in the clear room. Lipid emulsions were started on the second day of life as 1 g/kg per day and increased by 1 g/kg per day up to 3 g/kg per day and given as 24 hour infusion seperately from intravenous Primene® 10%, (provides a mixture of essential and nonessential amino acids as well as taurine and cysteine), glucose, electrolyte, vitamins and trace element solutions of PN. Enteral feeding was started on second day of life and increased by 10–20 cm^3/kg/day while PN volume was decreased, maintaining 40% of nonprotein calorie from lipid emulsion. Gestational age, birth weight, sex, Apgar scores, weight at 14th day of life, complications of prematurity such as respiratory distress syndrome, necrotizing enterocolitis, bronchopulmonary dysplasia, sepsis, retinopathy of prematurity were noted. Plasma lipid concentrations and acylcarnitine profile were evaluated at 14th day of life.

Metabolic status of the patients were evaluated by tandem mass spectrometry (API 3200, Shimadzu, Applied Biosystems, USA) to evaluate acyl-carnitine status with some drops of blood taken to guthrie paper. Two milliliters of blood was taken from a peripheric vein to evaluate the plasma lipid profile of the patients. Lipid infusion was stopped 8 h before the sample taken to prevent false hyperlipidemia. Lipid profile consisted of cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipopprotein (VLDL). Liver function tests (ALT, AST, GGT) were also measured from the same blood. Both lipid profile and liver function tests were analyzed with Roche Hitachii Modular P800 Chemistry Analyzer, Japan.

3. Statistical analysis

SPSS 17.0 was used for statistical analysis. Student *t*-test was used for comparison of numerical variables. The results are expressed as means ± standart deviations (SD) for normally distributed variables and as percentages for categorical variables. We evaluated categorical values using the Chi-square test and Fisher's exact test (when the chi-square test do not hold due to low expected cell counts was used to compare these proportions in different groups). Non-parametric comparisons between the patient groups were made with the Kruskal–Wallis test. Differences for continuous variables between two groups were analyzed by Student-t or Mann–Whitney U tests according to distribution of data. A per-protocol analysis was performed for the statistical analyses of efficacy and safety.

4. Results

There were no statistical differences in the gestational week, birth weight, body weight at 14th day of life (Table 1).

Liver function tests analyzed at the 14th day of life were normal in each group. Plasma lipid concentrations analyzed at the 14th day of life are seen in Table 2. Total serum cholesterol, triglyceride, HDL, LDL were not statistically significant between the groups whereas level of VLDL was statistically lower in Group I ($p<0.05$).

Free carnitine levels were 15.73 ± 10.67 in Group I and 34.25 ± 22.18 μM in Group II ($p=0.012$) and hexanoyl carnitine levels 2.18 ± 2.10 in Group I and 0.38 ± 0.12 μM in Group II, respectively ($p=0.005$) (Table 3).

Plasma acylcarnitine levels based on lipid chain were shown in Table 3. Plasma medium chain acylcarnitine levels were significantly higher in Intralipid group. Plasma branched chain aminoacid levels were statistically insignificant between the groups.

Complications of prematurity such as respiratory distress syndrome, necrotizing enterocolitis, bronchopulmonary dysplasia and retinopathy of prematurity were statistically insignificant between the groups. There were differences between groups in aspect of sepsis, seven patients (35%) in group I and four patients (20%) in group II received antibiotic therapy because of clinical and laboratory sepsis but the difference was not statistically significant ($p=0.48$).

5. Discussion

Soybean based lipid emulsions have been used in parenteral nutrition of premature infants over many years and is not adequate to support the needs of a developing infant [19]. In contrast to soybean based

Table 1
Demographic data of the patients.

	Group I (Intralipid) (n = 20)	Group II (Clinoleic) (n = 20)	*p* value
Gestational age (week)	29.2 ± 3.5	30.3 ± 2.5	NS
Birthweight (g)	1252.5 ± 458	1300.2 ± 480	NS
5- minute Apgar score	7	7	NS
Gender (male : female)	12/8	9/11	NS
Body weight at 14th day of life (g)	1300.8 ± 479	1344.9 ± 458	NS
Respiratory distress syndrome (n)	14	15	NS
Intracranial hemorrhage (≥Grade 3) (n)	2	1	NS
Retinopathy of prematurity (n)	0	0	NS

NS: nonspesific.

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Table 2
Plasma lipid concentrations of the patients.

	Group I	Group II	*P* value
Triglyceride, mg/dl	85.5 ± 37.7	94.7 ± 45.6	NS
Cholesterol, mg/dl	95.1 ± 16.1	99.1 ± 23.1	NS
High density lipoprotein (HDL), mg/dl	45.4 ± 13	49.4 ± 11.5	NS
Low density lipoprotein (LDL), mg/dl	33.6 ± 11.4	46.6 ± 19.1	NS
Very low density lipoprotein (VLDL), mg/dl	22.1 ± 8.9	38.2 ± 11.7	$P<0.05$

lipid emulsions, olive oil based ones are rich in long chain monounsaturated fatty acid, oleic acid, which decreases the high intake of linoleic acid [14]. Different lipid emulsions are used for PN but their clinical effects and related complications are still controversial.

As a result of decreased capillary tissue mass, endothelial lipoprotein lipase which is the enzyme essential for hydrolizing the lipid particles of the solution is decreased in VLBW premature infants, leads decreased lipid clearance [20]. Care should be taken at the follow up of triglyceride levels of the infants. In our study, infants neither in soybean nor olive oil group had triglyceride levels over 200 mg/dl. This results pointed us that both two lipid solutions are safe in terms of hypertriglyceridemia. But VLDL carries cholesterol, triglycerides, phospholipids and cholesterol esters that are synthesized in liver. Those unsoluble lipid components can only be carried if they are packaged with VLDL. VLDL level was statistically lower in soybean oil based group and we suggest that lowering the levels of VLDL may be a way of hemostasis to keep the serum triglyceride levels within normal levels.

Fatty acids are converted to acylcarnitinies by acyl coenzyme A and they are carried into the mitochondria for oxidation. In our study, as carnitine was utilized during mitochondrial transmission of fatty acids, carnitine levels were decreased in soybean oil group. This results showed that lipid solutions containing only long chain fatty acids may affect the carnitine metabolism of VLBW infants. We evaluated the plasma levels of acylcarnitines which is a fair reflection of the intramitochondrial acyl-CoA status. Medium chain acylcarnitine levels were significantly higher in Intralipid group which shows defective mitochondrial transport and catabolism of these fatty acids whereas branched chain amino acids were normal.

The major limitation of our study was based on the randomization method based on per protocol. We analyzed the patients that fulfill the inclusion criteria at the 14th day of life. This method may decrease the reliability of our study. Further studies should be planned by advanced randomization methods.

In our study, the effect of lipid emulsions on sepsis was evaluated, and seven patients (35%) in soybean oil based lipid emulsion group and four (20%) in olive-oil based lipid group had sepsis attack during hospitalization. It was shown that n-6 polyunsaturated fatty acid leads immunsupression [1]. In our study we demostrated that hexanoyl carnitine levels were higher in soybean oil-based group. We suggest that higher levels of hexanoyl carnitine, reflecting defective mitochondrial transport of hexanoyl which leads immunsupression, may be the cause of higher sepsis risk in Group I. Although we did not prove statistical important difference, we should be aware of this clinical effect of lipid emulsions on infection while using in such premature infants who are immunsupressive. In our study, complications of prematurity such as respiratory distress syndrome, necrotizing enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity were similiar between the groups.

Table 3
Levels of free carnitine and hexanoil carnitine between the two groups (mean ± SD).

	Intralipid	Clinoleic	*p value*
Free carnitine, μM	15.73 ± 10.67	34.25 ± 22.18	0.012
Hexanoyl carnitine, μM	2.18 ± 2.10	0.38 ± 0.12	0.005
Short chain acylcarnitine, μM	14.5 ± 6.9	16.0 ± 10.9	0.621
Medium chain acylcarnitine, μM	0.92 ± 1.29	0.27 ± 0.14	0.031
Long chain acylcarnitine, μM	0.18 ± 0.12	0.29 ± 0.24	0.067

In conclusion triglyceride, cholesterol, HDL and LDL levels, liver function tests and complications of prematurity like retinopathy of prematurity were similar between two groups. Level of VLDL was statistically lower in soybean oil-based group and we suggest that lowering the levels of VLDL may be a way of hemostasis to keep the serum triglyceride levels within normal levels. We became aware of different fatty acid catabolism of the two lipid solutions by comparing the plasma acylcarnitine levels of the patients. At our best knowledge this is the sole study reporting the plasma acylcarnitine profile after parenteral nutrition by two different lipid emulsions in preterm infants. Higher medium chain acylcarnitine levels in Group I may be the result of inefficient transport into the mitochondria. We suggest that higher levels of hexanoyl carnitine, reflecting defective mitochondrial transport of hexanoyl which leads immunsupression, may be the cause of higher sepsis attacks with soybean based lipid emulsions.

Conflict of interest

We have no conflict of interest.

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