An investigation of the safety of a lipid emulsion in very-low-birth-weight infants based on cytokine levels

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Short title: Effects of a lipid emulsion in very low birth weight infants

Abstract

Objective To verify whether administering a lipid emulsion aggravates infections and inflammation.

Study design Very-low-birth-weight (VLBW, <1500 g) infants born at <32 weeks gestational age between October 2013 and October 2014 at Dokkyo Medical University Hospital (Mibu, Tochigi, Japan) were treated with or without intravenous nutrition with a lipid emulsion. Infants were excluded who had congenital abnormalities, could not receive intravenous nutrition because of a poor general condition, or their physicians judged the treatment as inappropriate for them. Lipid emulsion with purified soybean oil was initiated at 0.5 g/kg/day on postnatal day 1. The dose was increased to 1 g/kg/day, and then to 1.5 g/kg/day (maximum dose). Blood tests were performed before (day 1) and after (day 8) initiating lipid emulsion treatment. Interleukin (IL)-6, IL-8, monocyte chemotactic protein 1 (MCP-1), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), total bilirubin (T-Bil), direct bilirubin (D-Bil) and insulin levels were measured. Changes in the respiratory condition, amount of oxygen used, and phototherapy duration were investigated.

Results 17 treated and 15 untreated VLBW infants were enrolled. The levels of IL-6, IL-8, MCP-1, TNF-α, CRP, T-Bil, D-Bil and insulin on days 1 and 8; respirator or surfactant use; amount of oxygen used; and phototherapy duration were not significantly different between the two groups.

Conclusions Lipid emulsion treatment did not increase inflammatory cytokine levels or aggravate respiratory disorders. Lipid emulsions, if proven safe, could be administered to VLBW infants soon after birth, which may prevent extrauterine growth restriction and improve the prognosis of intellectual development.

Abbreviations: CAM, chorioamnionitis; CRP, C-reactive protein; EUGR, extrauterine growth restriction; FiO2, fraction of inspired oxygen; MCP-1, monocyte chemotactic protein 1; MFI, median fluorescence intensity; PT, phototherapy; SD score, standard deviation score; SGA, small for gestational age; TNF-α, tumor necrosis factor-α

Introduction

The number of very-low-birth-weight (VLBW) infants has increased in Japan, and advances in neonatal medical care have improved the prognosis[1]. However, postnatal growth restriction in these infants is a major issue[2], with the most important risk factor being cumulative insufficient nutrient intake immediately after birth[3]. Such insufficient nutrient intake inhibits the growth of the head circumference and results in a low intelligence quotient (IQ) from school age onward[4]. Therefore, it is important to ensure proper nutrition soon after birth since this is a significant period of growth for the central nervous system (CNS).[5]

Enteral nutrition is difficult to administer to VLBW infants, and intravenous nutrition is needed to prevent a deficiency of fatty acids, which are essential for the development of the CNS. Intravenous lipid emulsions are routinely administered in Western countries, but omega-3 polyunsaturated fatty acids are not commonly used in Japan and the administration of intravenous lipid emulsions is avoided because of concerns that the aggravation of respiratory conditions and infections may lead to a poor vital prognosis. Lipid emulsions used in Japan are primarily soybean oil-derived omega-6 polyunsaturated fatty acids, which may affect immune function through several mechanisms, including influences on lymphocyte and macrophage functions[6].

Enlarged adipocytes induce insulin resistance and inflammation through several mechanisms. These include elevation in the level of inflammatory adipocytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor-α (TNFα), whereas the production of anti-inflammatory adipocytokines such as adiponectin is decreased in obese adipose tissue[7]. In obese patients, an acute-phase protein, C-reactive protein (CRP), is increased in the liver and at local sites because of the influence of inflammatory cytokines[8]. In obese adipose tissue, increased macrophage

infiltration due to inflammatory changes may lead to a collapse of regulation of adipocytokine production[8,9]. In addition, omega-6 polyunsaturated fatty acids have been implicated as a cause of cholestatic liver failure[10].

In this study, we measured IL-6, IL-8, MCP-1, TNFα, CRP, T-Bil, D-Bil and insulin levels and compared clinical symptoms before and after administering lipid emulsions in VLBW infants. We aimed to test the hypothesis that the administration of a lipid emulsion aggravates infection and inflammation.

Subjects and Methods

A prospective cohort study was performed in VLBW infants (<1500 g) born at <32 weeks gestational age between October 2013 and October 2014 at Dokkyo Medical University Hospital (Mibu, Tochigi, Japan). Written informed consent for voluntary participation in the study was obtained from the parents. The study protocol was approved by the Clinical Research Ethics Board of the Dokkyo Medical University (Mibu, Tochigi, Japan, registration number: 25052). Patients were randomly allocated alternately to the group with intravenous lipid emulsion treatment (i.e., study group) or the group without intravenous lipid emulsion treatment (i.e., control group). Infants with congenital abnormalities and major anomalies, or who were untreatable with intravenous nutrition because of a poor general condition, or who had severe complications were excluded. The total intake (g/kg) of glucose, protein, and lipids through drip infusion and tube feeding on postnatal days 0–7 (i.e., an 8-day period) was compared between the groups.

Treatment with a 20% lipid emulsion (20% Intralipid; Fresenius Kabi, Tokyo, Japan) with purified soybean oil (0.5 g/kg/day) was initiated at 17–34 h after birth. The daily dose was increased to 1 g/kg/day, and then to a maximum dose of 1.5 g/kg/day, based on the

Guidelines for Intravenous Nutrition in Japan (third edition)[11]. The syringe and route of administration were protected from light. Glucose administration was started at 4–8 mg/kg/min and the dose was adjusted, based on the blood glucose level. Amino acid administration was started at 0.5–1.0 g/kg/day and was increased by 0.5 g/kg/day up to a maximum dose of 2.5 g/kg/day, based on the same guidelines.

Enteral nutrition was initiated within 48 h after birth, and the starting amount is based on 10 mL/kg/day, then began to increase over 10 mL/kg/day by a few days. The content of glucose, protein, and lipid was calculated at 5 g/100 mL, 2 g/100 mL, and 3 g/100 mL, respectively, in breast milk[12] and at 7 g/100 mL, 1.5 g/100 mL, and 3.5 g/100 mL, respectively, in artificial milk.

Blood tests were performed before (i.e., day 1) and after (i.e., day 8) lipid administration. The IL-6, IL-8, MCP-1, TNFα, CRP, total bilirubin (T-Bil), direct bilirubin (D-Bil), and insulin levels were measured. Respirator use on days 1–10, the need for surfactant, and the mean fraction of inspired oxygen (FiO_2) on days 1, 8, and 10 were compared between the groups. Antibiotics were initiated for infection in infants with a sudden CRP increase or a marked left shift of white blood cells. The requirement for antibiotics for the exacerbation of infection during the study period was also examined. Furthermore, the duration of phototherapy was investigated in both groups. The study was discontinued in infants with an aggravation in their respiratory and general conditions, based on the judgment of the attending physician.

Sample Treatment and Measurements

Blood was collected in the early morning on days 1 and 8, and immediately centrifuged at 13,000 *g* at 4°C for 15 min. The levels of CRP, T-Bil, and D-Bil were measured in a

commercial laboratory. Residual serum was distributed to tubes for protein analysis and stored at –80°C within 2 h.

The sample was thawed and mixed by inversion to prepare a homogenous solution. It was then divided into 55-μg aliquots, followed by centrifugation at 13,000 *g* at 4°C for 5 min. A Milliplex MAP Human Adipokine Magnetic Bead Panel 2 kit (Merck Millipore, Darmstadt, Germany) was then used to measure IL-6, IL-8, MCP-1, TNFα and insulin levels. Measurements were duplicated for each sample using two wells.

The median fluorescence intensity of samples was measured using the Luminex 200TM System (Luminex Corporation, Austin, TX). The analysis software DNASIS Plex ver. 2.5 (Hitachi Solutions, Tokyo, Japan) was used to prepare a calibration curve. The concentration of the target protein in each sample was then calculated.

Amount of Oxygen Used

The FiO_2 was measured on days 1, 8, and 10, and the mean value was calculated.

Statistical Analysis

For numerical variables, values lower than the detection limit were regarded as zero. A Wilcoxon rank sum test was used without the assumption of a normal distribution. The Fisher exact test was used for categorical variables.

Results

A total of 37 VLBW infants were enrolled in the study. One infant was excluded immediately after birth because the parents did not give consent. Attending physicians randomly allocated the infants to the group that was administered the lipid emulsion (study

group, initially n=19) or was not administered the lipid emulsion (control group, initially n=17). In the study group, intracranial bleeding was detected on ultrasonography of the head at birth in one patient, and this infant was later excluded because of a poor general condition. Lipid administration was discontinued on day 1 in another patient because of a poor general condition associated with necrotizing enteritis, causing this patient to be withdrawn from the study. In the control group, one infant was excluded on day 8 because blood could not be collected, and one infant was excluded on day 8 because the blood sample volume was insufficient. Thus, 32 infants (study group, n=17; control group, n=15) were analyzed in the study (Table I).

There were no significant differences between the two groups in gestational age, gender, birth weight and height, each standard deviation (SD) score, or in the rate of small for gestational age (SGA) infants (P=1.0). The incidences of severe chorioamnionitis (CAM) of grade 2 or greater were 17.6% and 40% in the study and control groups, respectively, but this difference was not significant (P=0.24). There were also no significant differences between the two groups in the cord blood immunoglobulin M (IgM) level (P=0.31), the sugar or Amino acids dose in intravenous nutrition on days 0–7 (glucose, P=0.73; Amino acids, P=0.39), and the sugar, protein, or lipid dose in enteral nutrition (glucose, P=0.82; protein, P=0.97; lipid, P=0.57). There was also no significant difference in the dose of enteral nutrition per body weight (mL/kg/day) or breast feeding rate at 8 days after birth between the study and control groups.

Biochemical tests on day 1 showed no significant difference between the groups in any inflammatory cytokine level (IL-6, P=0.43; IL-8, P=0.52; MCP-1, P=0.28; TNFα, P=0.95) or in the CRP level (P=0.91) (Table II), or T-Bil and D-Bil (P=0.67, P=0.65) (Figure I), or insulin level (P=0.27) (Figure I). Biochemical tests on day 8 (after lipid emulsion

administration) similarly showed no significant differences between the groups in the levels of IL-6 (P=0.47), IL-8 (P=0.47), MCP-1 (P=0.14), TNFα (P=0.23), CRP (P=0.93), T-Bil and D-Bil (P=0.22 and P=0.31), and insulin (P=0.34) (Figure I).

Respirator use did not differ significantly between the groups during the study (P=0.15) (Table II). Surfactant treatment was required for 29.4% and 40% of infants in the study and control groups, respectively, but these rates were not significantly different (P=0.71) (Table II). The FiO_2 before (day 1) and after (day 8) the administration of the lipid emulsions did not differ significantly between the study and control groups (0.26±0.06 vs. 0.25±0.03, P=0.82; 0.24±0.01 vs. 0.23±0.02, P=0.47), and there was also no significant difference on day 10 (P=0.43). There was no significant difference between the two groups in the duration of phototherapy(P=0.98).

Discussion

There has been an increase in the number of VLBW infants in Japan and the prognosis of these infants has improved with advances in neonatal medical care. For VLBW infants in whom enteral nutrition is difficult, administering intravenous lipid emulsions soon after birth is important because the intake of lipids is required for the growth of the CNS. However, this treatment is only given to some VLBW infants in Japan because of the concern that the lipid emulsion will aggravate respiratory conditions and inflammation in premature infants.

High levels of inflammatory cytokines are found in infants born at an early gestational age[13] and show an association with gender[14]. Small for gestational age infants have high levels of inflammatory cytokines such as IL-8, but no significant changes in IL-6 or TNF-α[15]. High IL-8 levels may be correlated with bronchopulmonary dysplasia severity in SGA infants[16], and cytokines may also be related to prenatal infection[17]. The current study is the

first to examine changes in cytokine levels with intravenous lipid emulsion treatment soon after birth in VLBW infants. The absence of significant differences in inflammatory cytokines, CRP, T-Bil, D-Bil, and insulin levels on day 1 (i.e., before the lipid emulsion administration) showed that the infants that did and did not receive lipid emulsion had similar background factors of gestational age, gender, SGA, and CAM rates, and history of intrauterine infection.

Lipid administration during the first 21 days is significantly associated with the development of chronic lung disease[18], and an increased incidence of chronic neonatal lung disease has been attributed to the early administration of intravenous lipid emulsions in ventilated preterm neonates[19]. High levels of IL-6, IL-8, MCP-1, and TNF-α are also likely to cause bronchopulmonary dysplasia[20]. An increase in insulin has also been found after the intravenous administration of lipid emulsions in LBW infants[21]. However, in our study, there were no significant differences in inflammatory cytokine (i.e., IL-6, IL-8, MCP-1, and TNF-α) levels, jaundice level, and insulin levels before (day 1) and after (day 8) lipid emulsion administration in the treated and untreated groups.

Some infants with aggravation of respiratory conditions required the initiation of antibiotic treatment or a switch of antibiotics to treat infectious disease-induced aggravation of inflammation after the administration of lipid emulusions; however, this rate did not differ significantly between the two groups. There was also no significant increase in the amount of oxygen used on days 1 through day 10 with lipid administration, which suggests that administrating intravenous lipid emulsions soon after birth does not aggravate respiratory conditions or induce infectious disease by increasing inflammatory cytokines. This finding is in contrast to the concept that these conditions in preterm infants are aggravated by a lipid emulsion in the early postnatal period.

In one study[22], growth and development at 18 months old were markedly promoted in infants administered an increased amount of calories using Intralipid for 1 week after birth. Administering an intravenous lipid preparation may be useful in preventing essential amino acid deficiencies and maintaining the calorie dose for 1 week after birth with a limited total water volume that is administrable to VLBW infants for whom enteral nutrition is difficult to proceed immediately after birth.

Aggressive intravenous nutrition initiated early postnatally in VLBW infants improves head circumference development[23,24]. However, in our patients, the change in the weight (P=0.61) and head circumference SD score (P=0.53) between birth and term did not differ significantly between the two groups. The safety of drugs differs between races, and overseas guidelines cannot be used for Japanese patients. Thus, investigations at a higher dose are necessary to establish the safety of recommended lipid emulsions in Japan. This would allow improved nutrition soon after birth and lead to better head circumference growth.

Autoxidation of a lipid emulsion occurs upon exposure to oxygen and light (particularly short wavelength ultraviolet light), and reactive oxygen species induce a chain reaction of lipid peroxidation of unsaturated fatty acids, which reduces cell function and damages tissue[25]. However, hydrogen peroxide (i.e., peroxidase) production in a lipid emulsion can be reduced by protecting the emulsion from light and adding vitamins (C, B_2 and E)[26-28]. This shows the need to pay attention to the administration method. We protected the lipid emulsion during administration, which may have prevented peroxidation, and the administration of vitamin preparations may further prevent peroxidation. The omega-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid are unlikely to be oxidized. D'Ascenzo et al.[29] and Vlaardingerbroek et al.[30] found that the administration of fish oil to VLBW infants is safe and improves inflammation and nerve growth. The omega-6/omega-3

ratio may be important in future studies of lipid preparations. However, only omega-6 lipid preparations are approved for administration to VLBW infants in Japan. Thus, devising an administration method for lipid emulsions and examining fish-derived lipid emulsions are future tasks.

There are several limitations of this study. First, the small population of only 32 infants made it difficult to detect significant differences. Second, the dose of the lipid emulsion was determined using general guidelines in Japan, but was slightly lower than that in other guidelines (2–-3 g/kg/day[31,32] or 3–4 g/kg/day[33]). Third, only a lipid emulsion derived from soybeans was used, and a further study of fish-derived lipid emulsions is needed in more patients. Fourth, the investigation of SGA infants was limited by the small number of patients.

Despite these limitations, the study is important as the first description of changes in cytokine levels after lipid administration in VLBW infants soon after birth. Intravenous nutrition soon after birth is essential for preventing postnatal growth restriction in these infants and ensuring the safety of this treatment is clinically important. Our results showed that lipid emulsions induced no significant increases in inflammatory cytokine levels or aggravation of respiratory disorders or worsening of cholestatic jaundice in VLBW infants soon after birth. Establishment of the safety of lipid emulsions in these infants will allow this treatment to be administered soon after birth with the subsequent prevention of postnatal growth restriction and probable improvement of the prognosis for intellectual development. Further study is needed in this field in the future.

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Table I. Clinical characteristics of 32 very-low-birth-weight infants

Characteristics	Study group (n=17)	Control group (n=15)	*P*
Gestational age (d)	200±10	190±21	0.062
Birth weight (g)	1018±222	848±284	0.092
Birth weight SD score	−1.03±1.11	−1.01±1.10	0.94
Birth length (cm)	34.8±2.8	33.0±3.4	0.15
Birth length SD score	−1.07±1.13	−0.76±1.10	0.25
Boys, no. (%)	11 (64.7%)	6 (40.0%)	0.28
Small for gestational age infants, no. (%)	7 (41.1%)	5 (33.3%)	0.72
Cesarean section procedures, no. (%)	12 (70.5%)	8 (53.3%)	0.46
Apgar score at 1 min	4±1	3±1	0.24
CAM grade ≥2, no. (%)	3 (17.6%)	6 (40.0%)	0.24
IgM (mg/dL) of umbilical cord blood	5±2	7±4	0.31
I.V. intake to 8 days after birth			
Glucose (g/kg)	53.1±15.7	48.9±17.7	0.73
Amino acids (g/kg)	10.6±3.5	8.9±5.1	0.39
Lipid (g/kg)	7.3±1.7	0	<.0001
Enteral intake to 8 days after birth			
Glucose (g/kg)	12.5±9.9	14.7±10.9	0.82
Protein (g/kg)	2.9±2.3	3.2±2.5	0.97
Lipid (g/kg)	6.8±5.2	8.4±5.6	0.57
Enteral nutrition at 8 days after birth (mL/kg/day)	65.1±34.8	62.3±40.1	0.94
Breastfeeding rates at 8 days after birth (%)	65.5±38.4	81.6±29.7	0.24

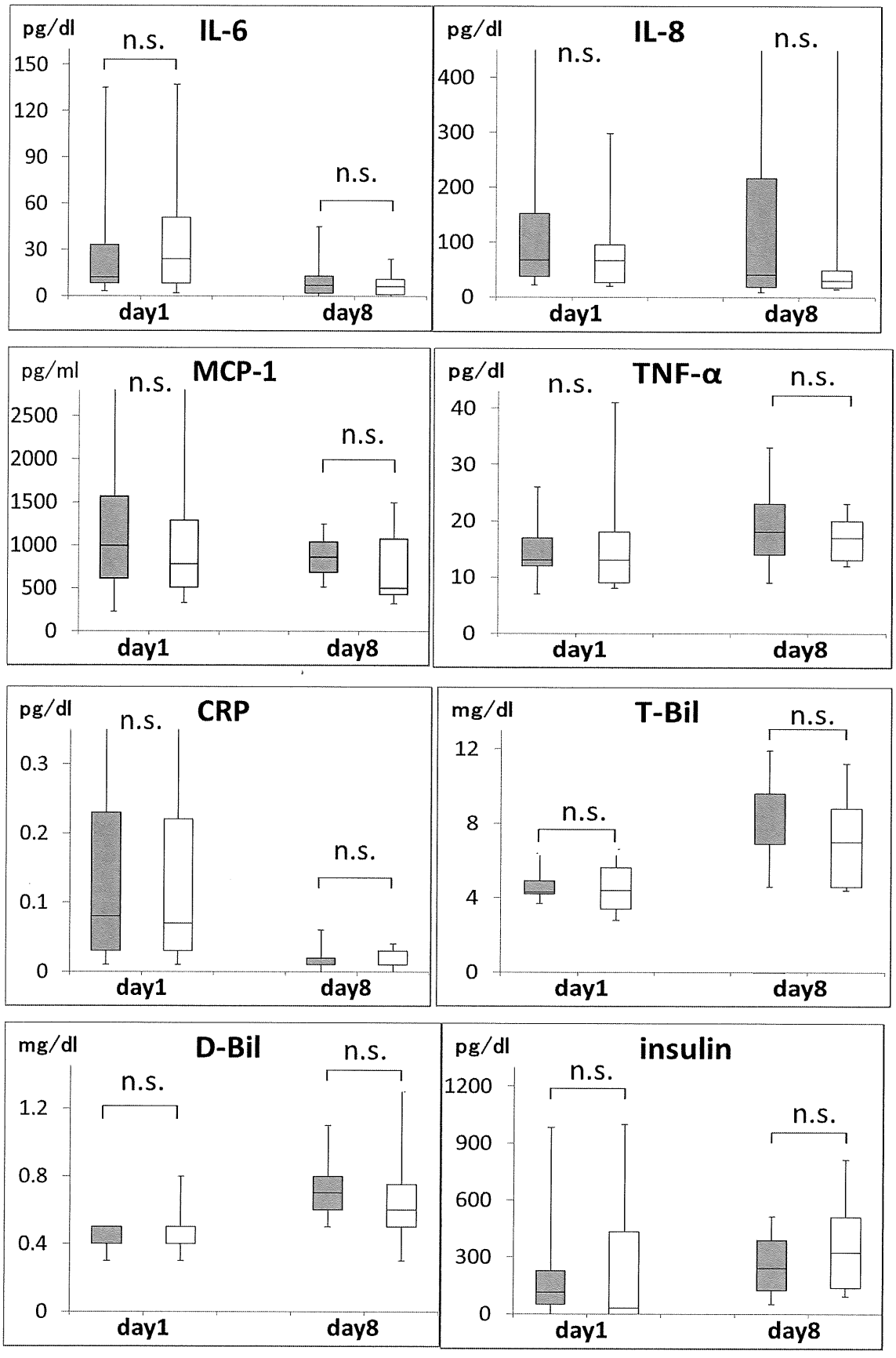
Data are presented as the mean ± standard deviation, unless otherwise indicated.

CAM, chorioamnionitis; IgM, immunoglobulin; I.V., intravenous; SD, standard deviation

Table II. Comparison of the clinical data before and after the administration of the lipid emulsions.

Characteristics	Study group (n=17)	Control group (n=15)	*P*
Respiratory disturbance			
Supplemental oxygen, day 1, FiO_2	0.26±0.06	0.25±0.03	0.82
Supplemental oxygen, day 8, FiO_2	0.24±0.01	0.23±0.02	0.47
Supplemental oxygen, day 10, FiO_2	0.24±0.02	0.23±0.02	0.43
Required surfactant rescue, no. (%)	5 (29.4)	6 (40.0)	0.71
Required mechanical ventilation, no. (%)	5 (29.5)	9 (60.0)	0.15
Infection			
Antibiotic therapy, no. (%) (excludes prophylactic treatment)	2 (11.7)	1 (6.6)	1.0
Duration of phototherapy (days)	1.9±0.9	1.9±0.8	0.98

There is no significant difference in the amount of oxygen used or the rates of surfactant and respirator use before (day 1) and after (day 8) the administration of the lipid emulsion. By day 10, there is no significant difference between the groups in the rate of infants requiring the initiation of antibiotic treatment or a switch in antibiotics for the aggravation of an infection. There was no significant difference between the two groups in the duration of phototherapy. Data are presented as the mean fraction of inspired oxygen (FiO_2) ± standard deviation or as the number (percentage).


pg/dl
IL-6
n.s.
n.s.
150
120
90
60
30
0
day1
day8
pg/dl
IL-8
n.s.
n.s.
400
300
200
100
0
day1
day8
pg/ml
MCP-1
n.s.
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TNF-α
n.s.
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pg/dl
CRP
n.s.
n.s.
0.3
0.2
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day1
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mg/dl
T-Bil
n.s.
n.s.
12
8
4
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day1
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mg/dl
D-Bil
n.s.
n.s.
1.2
0.8
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0
day1
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pg/dl
insulin
n.s.
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1200
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300
0
day1
day8

Figure 1. Comparison of biochemical data before and after administration of the lipid emulsion.

There were no significant differences between the groups in the levels of interleukin (IL)-6, IL-8, monocyte chemotactic protein 1 (MCP-1), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), total bilirubin (T-Bil), direct bilirubin (D-Bil) and insulin before (day 1) or after (day 8) administration of the lipid emulsion.

The minimum value, first quartile, median, third quartile, and maximum value are shown.

■ Study group

□ Control group

CRP, C-reactive protein