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Enclosed is a copy of a manuscript, Pulmonary Vascular Lipid Deposition After Administration of Intravenous Fat to Infants, which reports the relationship between pulmonary vascular lipid deposition, intralipid therapy, and serum triglyceride levels in newborn infants. The pulmonary histopathology which forms the basis of the report was supported by the Moran Foundation. This manuscript has been submitted for publication and is currently in the review process.

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PULMONARY VASCULAR LIPID DEPOSITION AFTER ADMINISTRATION OF INTRAVENOUS FAT TO INFANTS

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ABSTRACT

A review of the clinical course and pulmonary histopathology of 39 newborn infants who died during a 2-year period was made in order to determine whether pulmonary vascular deposition of lipid was seen in infants who had not received intravenous fat emulsion and to determine the relationship between serum triglyceride levels and the presence of pulmonary vascular lipid deposition. The use of fat emulsion was found to be associated with pulmonary vascular lipid deposition ($P < 0.02$). There was a positive correlation between the duration of fat emulsion administration and the severity of pulmonary vascular lipid deposition ($P < 0.05$). No relationship was seen between peak serum triglyceride level and frequency of elevated triglycerides and pulmonary vascular lipid deposition. Although fat emulsion administration was a risk factor for the development of pulmonary vascular lipid deposition, some infants who had not received intravenous fat emulsion had pulmonary vascular lipid deposition.

The use of intravenous fat emulsions has become routine to prevent essential fatty acid deficiency and to provide energy for neonates who receive total parenteral nutrition. The use of fat emulsions has aroused concern because of their potentially deleterious effect on pulmonary function. Results of previous studies have suggested that the use of intravenous fat emulsions in neonates is associated with the deposition of fat globules in pulmonary arteries[1,2], capillaries[1,3,4], and macrophages[1,2,4].

The interpretation of data from these studies is limited by the small number of subjects[1-4], the lack of a control group[1,2,4], and the difficulties involved in localizing intrapulmonary lipid on frozen sections of formalin-fixed lung. In addition, no attempt has been made to determine whether serum triglycerides correlate in any way with intravascular lipid deposition.

The aims of our study were 1) to determine whether pulmonary vascular deposition of lipid was seen in infants who had not received fat emulsions and 2) to examine the relationship between serum triglyceride levels and the presence of pulmonary intravascular lipid deposition.

SUBJECTS

Necropsy data were evaluated on all neonates who survived at least seven days (n = 39) and died during their initial hospitalization between January 1981 and January 1983. The charts were reviewed retrospectively for clinical and laboratory data.

METHODS

The lungs were inflated with formalin at a continuous distending pressure of 25 cm H_2O for at least 24 hours and were then cut, sectioned, and retained in formalin. The formalin-fixed tissue was stained with oil-red-O. A modification of the usual procedure was utilized, in that the tissue was bathed in sucrose solution before it was frozen. This method allowed thinner sections to be cut and thus permitted accurate localization of lipid droplets[5].

The hematoxylin- and eosin-stained sections of lung were reviewed by a microscopist to determine the type of pulmonary disease present. Using the oil-red-O stained sections, two 1-cm^2 sections were subsequently examined for the presence of vascular-associated lipid. The quantity of lipid found was graded according to the following schema: 0 (0-1 droplet/frozen section), 1+ (2 to 5), 2+

(6 to 10), 3+ (10 to 20), 4+ (>20). The sections of lung were examined without the knowledge of the clinical courses of the infants.

The nursery management of all infants was similar. Lipid administration was begun during the first 2 weeks of life. Lipids were infused continuously over 24 hours and triglyceride levels were measured routinely four hours after the start of the infusion or with any increase in infusion rate. When an elevated value was found, the rate of fat infusion was decreased and another triglyceride determination was made to confirm the presence of a normal value. A value of 150 mg/dl was considered normal, which in our laboratory corresponds to the maximum recommended triglyceride level of 100 mg/dl[6]. Serum triglycerides were measured by an enzymatic method (ACA®, Dupont, Wilmington, DE). Intralipid® (Cutter Laboratories, Berkeley, CA) was the sole fat emulsion used during the study period.

Gestational ages were assessed according to the method of Ballard et al.[7]. Sepsis episodes were documented by positive blood and/or cerebrospinal fluid cultures. Sodium heparin (1 unit/ml) was added to all parenteral nutrition fluid.

STATISTICS

The data were not distributed normally. Results were analyzed using the Mann-Whitney test, Fischer exact test, and multiple linear regression as appropriate to the data. Results were expressed as median and range unless otherwise noted. A P value of < 0.05 was considered significant.

RESULTS

Twenty-six infants had received lipids (L) and thirteen infants had not received lipid infusions (NL). There were no differences between the two groups in birthweight, gestational age, the incidence of small for gestational age infants, the types of lung disease present, and the number of sepsis episodes (Table).

The infants in the NL group were significantly younger at the time of death than those in the L group. The lung disease diagnoses were similar between the NL and L groups. The mean time from death to post-mortem examination was similar for both groups and all subgroups (14 ± 8.1 hr, mean $\pm$ SD).

All infants in both groups had lipid in pulmonary macrophages, chondrocytes, and interstitial cells. Vascular-associated lipid deposition was present as

microembolic droplets, endothelial staining, or deposits in the vessel wall. In Group L, 12 infants had pulmonary microembolic lipid and 2 had endothelial lipid deposition. One of these infants demonstrated both microembolic lipid and lipid in the vessel wall. The incidence of pulmonary vascular-associated lipid deposition in the L group was significantly greater than that in the NL group ($P < 0.02$). Two infants in the NL group were found to have vascular-associated lipid in their lungs (Table). One infant had both microembolic lipid and lipid in the vessel wall and the other infant had lipid in the vessel wall only. The clinical morbidities (oxygen and ventilator requirements, parenteral and enteral feedings) of these two infants did not differ from those of the study population.

The grade of pulmonary vascular-associated lipid deposition in infants in the L group correlated with the duration of lipid infusion ($P < 0.05$, $r = 0.41$) and with the percentage of the infants' lives during which lipids were administered ($P < 0.001$, $r = 0.6$). There was no correlation between the grade of vascular-associated lipid deposition and the peak serum triglyceride level, frequency of elevated triglyceride levels, birthweight, gestational age, or type and severity of lung disease.

There were no clinical differences between the L subgroups (infants with and without pulmonary vascular-associated lipid) with respect to birthweight, gestational age, incidence of small for gestational age, or sepsis (Table). Similarly, no differences were observed between the subgroups with regard to the highest serum triglyceride level, the duration of lipid administration, and the time between the cessation of lipid administration and death (Table). One of 12 infants in the Group L subgroup with no pulmonary vascular-associated lipid and 3 of 14 infants in the Group L subgroup who had intravascular lipid had an elevated serum triglyceride level within 1 to 2 days of death ($P < 0.1$).

The variability within the L subgroup with vascular-associated lipid deposition in the frequency of elevated serum triglyceride levels was due to one infant who demonstrated elevated serum triglyceride levels after cessation of the lipid infusion. If this infant is excluded, the mean length of time that serum triglyceride levels were elevated in the two subgroups was similar (mean 1.3 vs 2.3 days).

DISCUSSION

Reports of the presence of pulmonary vascular-associated lipid deposits in infants who had received intravenous fat have raised concern about the use of fat emulsions in neonates[1-4]. Premature infants with lung disease would seem to be at particular risk, but because of their predisposition to the development of essential fatty acid deficiency, the use of fat emulsions is particularly important in their treatment. In addition, when fluids are restricted, fat is an attractive energy source because of its high energy density.

The incidence of pulmonary vascular-associated lipid deposition was greater in the L group. The etiology of the lipid deposition was unclear but appeared to be related to the duration of lipid administration. Two infants who had never received lipids, however, were found to have vascular-associated lipid deposition. This finding has been noted in other studies[3,8]. These results suggest that factors other than the administration of intravenous lipid may have caused or potentiated the deposition of pulmonary intravascular fat.

Acute overinfusions of intravenous lipid have been reported to cause the 'fat overload syndrome' which results in the intrapulmonary deposition of fat[9].

Consequently, we anticipated that intravascular lipid would be found in the lungs of infants who had had a long duration of lipid therapy, elevated serum triglyceride levels, or serum triglyceride levels which were elevated often.

Why serum triglyceride levels and the frequency of elevated triglyceride levels did not correlate with the intravascular deposition of lipid is unclear. Serum triglycerides may have been elevated at times when serum levels were not measured. In our clinical experience, however, serum triglyceride levels remain stable in patients whose clinical status is not deteriorating. Serum triglyceride levels are checked frequently in patients in our nursery whose clinical status has worsened. Another explanation for the lack of association between serum triglyceride levels and pulmonary vascular-associated lipid may be that the levels were not sufficiently high to cause consistent lipid deposition. Other variables which could not be examined in this study, such as the amount of glucose administered, may have altered the threshold for intravascular lipid deposition in some patients. Thus, the results should not be interpreted to suggest that serum triglyceride levels should not be monitored. At the triglyceride levels found in the present study, the duration of lipid therapy was more important in the development of pulmonary vascular-associated lipid deposition than was the frequency of elevated serum triglycerides. Indeed,

a lack of association between elevated serum triglycerides and lipid deposition may have been the result of the frequent monitoring of serum triglycerides, precluding pulmonary vascular-associated lipid deposition in infants who received lipids for a short period of time.

Authors of previous studies have suggested that vascular-associated lipid may be a post-mortem artifact[10] and may be related to the serum triglyceride level at the time of death[5]. Lipid emulsion is known to cause the production of an abnormal phospholipid[11]. Other authors have suggested that post-mortem changes in pH may alter these particles and cause them to coalesce, as appears to occur in the intact lipid emulsion chylomicron[10].

Because lipid administration was discontinued at least 3 days before death in one-half of the infants with pulmonary vascular-associated lipid and serum triglyceride levels were normal in almost all (22 of 26) of the group L infants at the time of death, it seems unlikely that instability of the chylomicrons or post-mortem artifact would account for the finding of vascular-associated fat. In addition, lipid deposition which occurs as a post-mortem artifact is diffuse and unlike the patchy distribution which was found in the present study[5]. Such explanations would not account for the two infants in group NL who had intravascular fat.

In conclusion, an increased incidence of pulmonary vascular-associated lipid deposition appeared to have been associated with lipid infusions, although it was also seen in infants who had not received lipid infusions. In the present study population, the duration of lipid administration was related to the severity of vascular-associated lipid deposition. The etiology of this finding and its clinical significance remain unclear. The occurrence of lipid deposition during life must be determined in order to assess the clinical implication of the post-mortem findings.

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Table 1. - Clinical and pathology summary of the study infants.

	Non-lipid Group (NL)		Lipid Group(L)	
Subgroup (n)	No Lesion (11)	Intravascular Lipid (2)	No Lesion (12)	Intravascular Lipid (14)
Birthweight (g)	1200(616-3470)	1680*; 2100*	1350(660-3760)	920(520-3520)
Gestation (wk)	31.0(23-41)	30; 34	29.5(26-40)	28.5(24-41)
Age at death (wk)	13.0(10-23)†‡	17; 44	24.0(11-139)†	35.5(13-166)‡
Small for gestational age (n)	4	AGA; SGA	1	2
Sepsis episodes (n)	3	Yes; No	2	5
Grade of intravascular lipid deposit	—	1; 2	—	1.0(0-4)
Highest triglyceride level (mg/dl)	—	—	175(104-369)	205(69-454)
Cases with elevated triglyceride (n)	—	—	7	10
Frequency elevated triglyceride (d)	—	—	1.0(0-4)	1.0(0-58)
Duration lipid infusion (d)	—	—	8.0(3-56)	13.5(1-55)
Duration from lipid cessation to death (d)	—	—	5.5(0-17)	2.0(0-73)

Except where indicated, the median and ranges are given.

* Data for the two infants in the NL group who had deposits of intrapulmonary fat are given separately in the corresponding columns.

† $P < 0.02$.

‡ $P < 0.0001$; for the combined L subgroups vs NL, $P < 0.0005$.