Pulmonary intravascular lipid in neonatal necropsy specimens

J W L Puntis, D I Rushton

Abstract
The lungs of 482 liveborn infants were examined at necropsy for the presence of intravascular lipid. Forty one patients had received parenteral feeding (including lipid emulsion in 30), and 441 had died before starting feeds or had received enteral feeds alone. Tissue was processed into wax and then stained with Sudan black; intravascular lipid was found in 15 of 30 infants who had received intravenous fat (Intralipid), but in no others. Those patients with positive lipid staining had received significantly more fat during parenteral nutrition than those in whom intravascular lipid was not found but the two groups were otherwise clinically indistinguishable. Using this staining technique intravascular lipid can be shown relatively often, although only in patients who have received intravenous lipid emulsion. The location of fat, predominantly in small pulmonary capillaries, and the absence of lipid emboli in other organs, suggests that lipid coalescence takes place before death and is not a postmortem artefact. The clinical relevance remains uncertain.

Although the occlusion of pulmonary capillaries by intravascular fat has been reported in necropsy specimens of newborn infants who were given parenteral nutrition the incidence, aetiology, and clinical importance of this are controversial. In one study, lung tissue containing intravascular lipid was found to be rich in linoleic acid, consistent with the lipid 'emboli' having been derived from the intravenous fat emulsion used during parenteral nutrition. Other workers have suggested that intravascular lipid is no more than postmortem artefact. The aims of this study, therefore, were to determine prospectively the incidence of pulmonary intravascular fat at necropsy in both enterally and parenterally fed infants, and to examine its association with parenteral feeding.

Patients and methods
INFANTS RECEIVING PARENTERAL NUTRITION
In a regional neonatal intensive care unit during a 10 year period (1980–9) 41 infants who had been parenterally fed came to necropsy. Their median (range) gestational age was 28 weeks (25–40) and weight 880 g (450–2820). The most common indication for parenteral nutrition was prematurity and failure to tolerate enteral feeding in association with severe hyaline membrane disease requiring ventilation (n=38); indications in the three remaining patients were necroting enterocolitis, ischaemic colitis, and ileal atresia. All patients received amino acid/dextrose solution (Vamin 9 glucose, KabiVitrum) and 30 had also been given an intravenous fat emulsion (Intralipid 10%, KabiVitrum). Routine biochemical monitoring of fat metabolism was not carried out other than for daily visual inspection of plasma for lipaemia. Lipid infusion rates varied between 0.5 g/kg/day and 3.0 g/kg/day, and the infusions were discontinued each morning four hours before blood sampling. Details of feed intake and clinical condition were obtained retrospectively from nursing and medical records.

INFANTS RECEIVING ENTERAL NUTRITION
In addition to those who had been parenterally fed, the lungs from 441 other live born infants were also studied for evidence of intravascular pulmonary fat. These patients had either been fed by the enteral route alone, or had died before any form of feeding was started.

PREPARATION OF TISSUE SPECIMENS
Necropsy specimens were prepared according to a standard procedure. One block of tissue was taken from each lobe of each lung and processed in wax using chloroform to remove neutral fat. Sections were then stained with Sudan black and examined for residual lipid. All specimens were examined by the same person (DIR) who did not know whether the parenterally fed infants had received Intralipid, or the amino acid/dextrose solution alone. Organs other than lungs were not routinely stained for fat by this method.

STATISTICAL ANALYSIS
Significance of differences were assessed by the Mann-Whitney U test or the χ2 test, and a probability of <0.05 was accepted as significant.

Results
INFANTS RECEIVING PARENTERAL NUTRITION
For the group as a whole, the median (range) duration of parenteral nutrition was 14 days (1–46). Pulmonary intravascular lipid was not found in any of the 11 patients given amino acid/dextrose mixtures alone, but was present in 15 (50%) of infants who had received Intralipid. Lipaemic serum had not been noted in any patients during feeding. In all cases, lipid was found in the pulmonary capillaries, usually towards the periphery of lobules, and in two
Patients receiving intravenous fat emulsion: comparison at time of death between those with and without pulmonary intravascular lipid in necropsy specimens. Values are expressed as median (range)

<table>
<thead>
<tr>
<th>Pulmonary intravascular lipid in necropsy specimens</th>
<th>Present (n=15)</th>
<th>Absent (n=15)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>880 (530-1890)</td>
<td>840 (450-2820)</td>
<td>0.88</td>
</tr>
<tr>
<td>Postconceptual age (weeks)</td>
<td>27 (25-34)</td>
<td>28 (26-38)</td>
<td>0.33</td>
</tr>
<tr>
<td>Age at death (days)</td>
<td>28 (8-54)</td>
<td>19 (4-84)</td>
<td>0.06</td>
</tr>
<tr>
<td>No of days that fat was infused</td>
<td>16 (1-35)</td>
<td>4 (1-28)</td>
<td>0.003</td>
</tr>
<tr>
<td>Total amount of fat given (g/kg body weight)</td>
<td>36 (11-112)</td>
<td>5.5 (1-49)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interval between death and necropsy (hours)</td>
<td>58 (9-120)</td>
<td>68 (27-120)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

of these lipid was also found in alveolar macrophages. One patient was strikingly different in that lipid was found in pulmonary arteries; myocardial, pancreatic, brain stem, and salivary gland capillaries; renal glomerular and medullary vessels; and the portal vein.

Ten of 15 patients who had lipid in their lungs and 12 of those who did not had received Intralipid infusions within 24 hours of death (p=0.88). Four patients with positive intravascular lipid staining had not been given Intralipid for between three and eight days before death. The clinical characteristics of the patients who received Intralipid are shown in the table. Patients in each category were similar except in respect of the amount of Intralipid received, which was significantly greater among those who had positive intravascular fat staining at necropsy.

From review of the clinical records it did not seem that alteration in the rate of lipid infusion was associated with subsequent deterioration in the clinical condition in any of the patients, although we did not analyse blood gas and ventilation data in relation to lipid infusion. Heparin was not included in feeding solutions but all patients received 3 units of heparin each hour with arterial flush solutions during part or all of the time they were receiving parenteral nutrition. There were identical proportions of small for gestational age infants among those in whom intravascular fat was and was not found.

INFANTS RECEIVING ENTERAL NUTRITION
None of the 441 infants who received enteral nutrition, or died before feeding could be started, had evidence of intravascular fat at necropsy.

Discussion
We have confirmed the previously described association between the giving of Intralipid and the presence of pulmonary lipid emboli at necropsy, using wax impregnation and Sudan black staining of lung tissue. Although we found intravascular fat exclusively in patients who had been given intravenous lipid emulsion (half), some studies using alternative tissue preparation and staining methods have shown occasional incidences of intravascular fat in patients who had not received Intralipid. This discrepancy probably relates to the different techniques used for diagnosing the presence of lipid. Processing tissue into wax removes neutral lipid so that fat that is seen after subsequent staining with Sudan black must be in a form that is relatively insoluble in chloroform. That this fat is Intralipid seems in little doubt; its vegetable origin and coating of egg phospholipid possibly make it less soluble than endogenous fat. Intravascular fat seen with Oil Red O in frozen sections of lungs from patients who have not received Intralipid may on the other hand be of endogenous origin, and this is probably a phenomenon quite different from the lipid emboli seen after parenteral nutrition. In one study that challenged the association between Intralipid and fat emboli lungs were fixed immediately after death and then stained with Sudan IV. This method has not been used by other workers and is less likely to show up Intralipid, as Sudan IV is not as lipid soluble as Sudan black.

Patients with intravascular lipid at necropsy had received significantly greater amounts of fat than those without (p<0.0001), which adds further support to the existence of a relationship between the use of Intralipid and the presence of fat emboli at necropsy. Lipid was infused at rates regarded as being metabolically well tolerated (<3 g/kg/day). We cannot comment on differences in lipid metabolism between the two groups as no monitoring other than inspection of the plasma for lipaemia was done, and no instances of lipaemia were recorded. Visual inspection of plasma is, however, an unreliable way of predicting lipid profiles. We did not observe lipid deposits in pulmonary arterial walls as some workers have described, although we have seen similar lesions in other patients who have received parenteral nutrition for many months.

Heparin given through arterial catheters could possibly affect lipid clearance by increasing the amount of lipases in the circulation; a standard concentration of heparin was used in arterial flush solutions, however, and intake in patients with and without intravascular fat was much the same. Although infants who are small for gestational age may clear lipid from the circulation more slowly than appropriately grown infants, there was no difference in the proportion of growth retarded patients with and without positive fat staining.

The observation that lipid is usually limited to small pulmonary capillaries and not found throughout the entire vascular system suggests a filtering out process taking place before cessation of circulation. The assertion that intravascular lipid is merely a postmortem artefact would therefore seem implausible, although this could apply to our one patient with lipid seen in many different organs. Because most Intralipid droplets are of similar size to endogenous chylomicrons (mean diameter 0.15 μm), before emboli can lodge in the pulmonary capillaries (8–12 micron diameter) the droplets must coalesce for some reason. Coalescence or 'creaming' of Intralipid occurs in vitro in the...
presence of C reactive protein,\textsuperscript{11} and fat embolism has been described in one infant with a high plasma concentration of C reactive protein.\textsuperscript{12} Many preterm infants die of multisystem failure, sometimes with infection as an additional complication, and it is probable that C reactive protein concentrations are often raised.\textsuperscript{13} Recent evidence, however, casts doubt on the relationship between creamping and C reactive protein concentrations.\textsuperscript{14}

As lipid emboli were found in some patients who had not received any Intralipid for several days before death (over a week in two cases), it is possible that once emboli have formed they may remain relatively resistant to degradation. Although there is little evidence to suggest that fat microembolisation in itself is harmful to the patient,\textsuperscript{15} under certain circumstances high infusion rates of lipid have been associated with falls in arterial oxygen saturation.\textsuperscript{16} Whereas it is tempting to think that this may be caused by lipid microemboli blocking pulmonary capillaries and thereby altering perfusion ventilation ratios, there is evidence that an alternative mechanism exists through which fat leads to increases in concentrations of vasodilating prostaglandins that unblock hypoxic vasoconstriction and thereby increase intrapulmonary right to left shunting.\textsuperscript{17} In none of our patients was any such clinical effect commented on in the medical records.

Our data suggest that lipid microemboli may be common during administration of intravenous lipid emulsion but are probably of little clinical relevance. Caution in the use of Intralipid is advisable in those infants at risk of increased pulmonary vascular resistance,\textsuperscript{18} but anxiety about lipid emboli should not preclude the use of lipid emulsions in preterm infants who cannot tolerate enteral feeding.

We thank Dr GM Durbin and Dr MEI Morgan for help with this study.