Unbound Free Fatty Acids from Preterm Infants Treated with Intralipid Decouples Unbound from Total Bilirubin Potentially Making Phototherapy Ineffective

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Abstract
Extremely low birth weight (ELBW; <1,000 g) infants have poor outcomes, often compromised by bilirubin neurotoxicity. We measured unbound bilirubin (B_un) and unbound free fatty acid (FFA) concentrations in preterm infants treated with Intralipid. Intralipid lipolysis produces FFA that decouples unbound bilirubin from total bilirubin, making phototherapy ineffective. Extremely large unbound FFA concentrations from Intralipid lipolysis are themselves potentially lipotoxic.

Key Words
Extremely low birth weight infants · Outcome · Intralipid · Unbound free fatty acids · Unbound bilirubin · Phototherapy · Indomethacin · Ibuprofen

Established Facts
- Extremely low birth weight (ELBW) infants have poor outcomes; approximately 50% die or have severe neurologic deficits.
- Aggressive phototherapy has not substantially improved these outcomes.

Novel Insights
- Most ELBW infants are treated with Intralipid and the unbound free fatty acid (FFA) produced from Intralipid decouples unbound bilirubin from total bilirubin.
- As a consequence, phototherapy may be ineffective in Intralipid-fed ELBW infants because unbound bilirubin will remain at toxic levels even when total bilirubin is lowered well below aggressive phototherapy targets.
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Fatty acid (FFA_u) levels in 5 ELBW infants in a trial examining the effects of pharmacologic ductal closure on infants treated with Intralipid infusion (3 g/kg/day). The levels for all infants (mean ± SD) were: total serum bilirubin (TSB) 4.6 ± 1.7 mg/dl, FFA_u 376 ± 496 nM, and B_f 42 ± 30 nM. Of the 3 infants who died, 2 had TSB <5.9 mg/dl but FFA_u >580 nM and B_f >75 nM. Multiple regression revealed a major effect on B_f levels due to FFA_u, indicating that Intralipid elevated levels of FFA_u and B_f. Indomethacin or ibuprofen reduced B_f levels, most likely by reducing FFA_u levels through lipase inhibition. Because displacement of B_f by FFA_u decouples B_f from TSB, phototherapy may not reduce the risk of bilirubin or FFA_u toxicity in Intralipid-treated ELBW infants.

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Introduction

Extremely low birth weight (ELBW; <1,000 g) infants have poor outcomes; approximately 50% of these infants die or have severe neurologic deficits [1, 2]. In an effort to improve these outcomes, the NICHD Neonatal Research Network assigned 1,974 ELBW infants to either aggressive or conservative phototherapy, but found that aggressive phototherapy did not improve outcomes substantially [1]. Most ELBW infants receive parenteral nutrition that includes infusion of lipid emulsions (Intralipid). Previous studies have demonstrated that Intralipid increases plasma free fatty acid (FFA), which in turn increases unconjugated bilirubin (B_f) by displacing bilirubin from albumin [3–5]. In this small pilot study, we sought to determine how Intralipid infusion affects unconjugated FFA (FFA_u) and B_f levels in ELBW infants, and whether these levels were correlated with outcome (death). These are the first measurements in Intralipid-treated infants of both FFA_u and B_f, using a newly developed sensor [6].

Case Report

Five male ELBW infants, 3 African Americans and 2 Caucasians, were delivered by C-section and admitted to the level III NICU of Cooper University Hospital. Each was treated with Intralipid infusion (up to 3 g/kg/day) and participated in a trial examining the effect of pharmacologic ductal closure. No infant had sepsis when the FFA_u and B_f samples were obtained, but all had respiratory distress syndrome and one had an intraventricular hemorrhage (patient No. 4). Infants were selected if the clinician decided that pharmacologic ductal closure was required and informed consent was obtainable from the parent. The institutional review boards of Cooper University Hospital and Torrey Pines Institute for Molecular Studies approved this investigation. Four infants were treated with indomethacin and one with ibuprofen. The mean (±SD) infant birth weight was 710 ± 155 g and gestational age was 27.0 ± 1.3 weeks; B_f and FFA_u were measured at 110 ± 26 h. All infants were treated with phototherapy.

Blood plasma samples of 50 μl were obtained before and within 2 h of drug treatment, and shipped with the patient information deleted to FFA Sciences for determination of B_f and FFA_u. Total serum bilirubin (TSB) was measured by Cooper Hospital using the diazo method. Phototherapy was carried out with Olympic Model 66 Bank and Mini Spot lights (Natus Inc.). Irradiance was monitored with an Olympic Model 22 light meter and maintained at approximately 15 μW/cm²/nm. Phototherapy was delivered continuously for periods ranging from 4 to 8 days and maximum TSB levels were <7.6 mg/dl. Student’s T tests and multiple regression analyses were performed with XLSTAT (AddinSoft), and p < 0.05 was considered significant.

FFA_u and B_f were measured using fluorescently labeled mutants of fatty acid binding proteins (probes) [6–8]. The B_f probe binds unconjugated bilirubin with high affinity (K_d = 16 nM), but is poorly sensitive to FFA (K_d >3,000 nM), conjugated bilirubin (K_d >300 nM), bilirubin photoisomers, bilirubin oxidation products, ibuprofen, and indomethacin [6]. The measurements simply require adding the probe to a plasma sample and measuring fluorescence at two emission wavelengths: 457 and 550 nm for the FFA_u probe, and 525 and 580 nm for the B_f probe (excitation = 375 nm for both). The ratio of fluorescence at the two wavelengths, together with probe characteristics, yielded the FFA_u and B_f concentrations. Fluorescence was measured at 22 °C using a handheld ratio fluorometer in which plasma sample volumes were 4 μl and 25 μl for the FFA_u and B_f measurements, respectively; and after dilution the total volume was 200 μl [6, 7].

The accuracy of the B_f probe measurements were confirmed by comparison with the peroxidase assay in adult plasma supplemented with bilirubin as well as in bilirubin-albumin in vitro measurements [6]. The B_f probe has an average coefficient of variation of 3% for repeated measurements of aqueous bilirubin, bilirubin–albumin complexes, and bilirubin-spiked adult plasma over a B_f range from 1 to >350 nmol/l [6]. Measurements with the B_f probe in contrast to the peroxidase method determines the B_f concentration of bilirubin IXa directly in a single measurement and is insensitive to interferents that can interfere with the peroxidase measurement [6].

All TSB values were <7.1 mg/dl, with a median of 5.3 and range of 2.1–7.1 (table 1). These levels were below the exchange transfusion threshold for ELBW infants [9]. FFA_u levels were exceptionally high in all cases, greatly exceeding the mean levels (1.5 ± 0.6 nM) found in healthy adults [10] as well as the 4.4 ± 1.7 nM found in the cord blood of hypoxic infants [11]. B_f levels were well correlated with FFA_u levels (p = 0.003), but not significantly with TSB (p = 0.06). In most cases, FFA_u and B_f levels decreased after indomethacin or ibuprofen administration. Importantly, of the 3 infants who died, 2 had the highest B_f and FFA_u levels. The putative causes of death were pseudomonas sepsis, staphylococcal sepsis and respiratory distress syndrome/persistent pulmonary hypertension for infants 1, 4, and 5, respectively. As the parents did not consent to autopsies, no neuropathologic evidence was obtained for bilirubin toxicity.
The 1981 AAP recommendation for the use of intravenous fat emulsions in pediatric patients raised concern that FFA generated by such infusions might displace albumin-bound bilirubin and thereby risk subjecting infants to bilirubin toxicity [12]. Although most subsequent studies observed increased FFA and B_f with fat infusion, other studies did not observe such associations [14, 15]. The principal cause for these disparate results is likely the greater frequency of FFA increases in infants with a gestational age ≤30 weeks [3–5, 13]. Additionally, most studies present FFA and B_f values averaged over the cohorts of infants. Such averaging may obscure the high degree of variability of FFA responses as is apparent in table 1.

Our results are consistent with previous studies showing that FFA produced from Intralipid in ELBW infants increased B_f with no change in TSB [4, 5]. In the presence of high levels of FFA, B_f and TSB are effectively decoupled because displacing relatively small fractions of TSB can dramatically increase B_f. Additionally, our study provides an estimate of the fraction of bilirubin that is displaced from albumin by FFA. This fraction is \( \frac{(B_f - B_f(0))}{TSB} \), where B_f and TSB are the measured values from table 1 and B_f(0) is B_f in the absence of FFA, approximately 10 nM (fig. 1). The displaced fractions for infants No. 1 and No. 5 were 0.07% and 0.13% of TSB, respectively. As a consequence, even if aggressive phototherapy reduced TSB to 2 mg/dl, the corresponding B_f for infants No. 1 and No. 5 would be reduced only to 24 and 45 nM, levels that may remain toxic for these extremely premature infants [16]. FFA from sepsis would further increase FFA_u and B_f levels would be increased further by low albumin [17, 18]. Thus, in the presence of Intralipid, phototherapy alone may not be effective for prevention of acute bilirubin encephalopathy.

The increase in FFA levels resulting from Intralipid infusion differ substantially in different infants and can
increase rapidly with increasing Intralipid concentration [4, 5]. Therefore, careful monitoring of $B_f$ while Intralipid levels are increased slowly may allow for Intralipid infusion without substantial bilirubin displacement. This possibility is apparent from the in vitro titration of albumin with FFA in the presence or absence of bilirubin (fig. 1). This shows, for a total bilirubin concentration of 2.3 g/dl (342 μM), that the $B_f$ concentration is 10 nM in the absence of FFA. Under these conditions, $B_f$ increases only gradually until the FFA-to-albumin ratio approaches 4.0 where $B_f$ increases exponentially. This suggests that by maintaining FFA-to-albumin levels below a critical threshold (in this case about 4.0) via titrating the level of Intralipid, $B_f$ may be maintained at nontoxic levels even in the presence of Intralipid.

In addition to effects on $B_f$, the FFA$_u$ levels produced from Intralipid may themselves be toxic. FFA$_u$ at lower levels than in infants No. 1 and No. 5 interferes with a variety of cellular functions including immune and cardiac functions [19, 20]. Limiting Intralipid may therefore reduce acute bilirubin encephalopathy as well as FFA$_u$-mediated toxicity.

Although ibuprofen may displace bilirubin from albumin, we found that FFA$_u$ and $B_f$ decreased following indomethacin and ibuprofen treatment for patent ductus arteriosus (table 1). Because FFA$_u$ and bilirubin are tightly coupled, this most likely reflects a reduction in FFA$_u$ due to inhibition of lipoprotein lipase by indomethacin and probably by ibuprofen [21]. Therefore, such drugs might also be used to reduce FFA$_u$ levels in infants receiving Intralipid to ameliorate the risks of acute bilirubin encephalopathy as well as FFA$_u$ toxicity.

References


