Lipid and lipoprotein concentrations markedly decline in patients with a variety of medical and surgical disorders.\(^1,2\) The mechanism for this hypolipidemia is likely related to the cytokines that mediate the systemic inflammatory response. Cytokines, such as interleukin-6 and tumor necrosis factor, are potent negative regulators of lipoprotein metabolism in vitro\(^3,4\) and in vivo in humans.\(^5,6\) Hypolipidemia may have important clinical consequences. Low lipid and lipoprotein levels have been associated with poor clinical outcomes\(^7-9\) and the development of infectious disorders in a study of 15,000 healthy men and women.\(^10\)

A mechanism by which hypolipidemia might adversely influence clinical outcomes is the ability of lipids and lipoproteins to bind and neutralize bacterial lipopolysaccharide (LPS).\(^11-13\) LPS binding with LPS-binding protein\(^14,15\) and the cell-surface CD14 receptor\(^16\) can trigger the Toll-like receptor\(^17\) and release an assortment of inflam-
Method

In contrast, binding of LPS to lipoproteins substantially decreases cytokine release.\textsuperscript{19-21} There is substantial evidence for an in vivo effect of the interaction between lipoproteins and LPS. Transgenic mice with elevated high-density lipoprotein (HDL-C)\textsuperscript{22} or low-density lipoprotein (LDL-C) cholesterol concentrations\textsuperscript{23} are resistant to endotoxin challenge. The administration of plasma-derived lipoproteins to human volunteers blocked many of the physiologic effects seen with infusion of LPS.\textsuperscript{24,25} We have shown that LPS neutralization correlates with the amount of phospholipid in the lipid or lipoprotein particles.\textsuperscript{26}

The use of plasma-derived lipids and lipoproteins as a treatment for endotoxemia is limited by the need for large quantities of source plasma and the inherent infectious risks in using a blood-derived product. A protein-free, phospholipid-rich emulsion offers the ability to bind and neutralize bacterial endotoxin without the risk of infection transmitted from blood-derived lipoproteins. We developed an emulsion containing 92.5\% of the lipid as soy phospholipid and 7.5\% as soy triglyceride emulsified in 18 mM sodium cholate. Preclinical studies using this emulsion in a pig peritonitis model of sepsis\textsuperscript{27} demonstrated a large survival advantage (p < 0.001) for emulsion-treated animals.\textsuperscript{28} The effective phospholipid level was determined to be approximately 300–500 mg/dL. Preclinical studies in the pig also demonstrated that the primary adverse effect was a reversible hemolytic anemia at doses that raised serum phospholipid levels above 2000 mg/dL (unpublished data).

We initiated a Phase I double-blind crossover study to determine whether the intravenous administration of a protein-free, phospholipid-rich emulsion was a safe and effective method for increasing blood phospholipid levels in human volunteers.

Methods

Population

Thirty healthy men aged 18–45 years were studied. Subjects were recruited from advertisements placed in local newspapers. Healthy subjects were defined as individuals who were free from significant cardiac, pulmonary, gastrointestinal, hepatic, renal, hematologic, neurologic, and psychiatric disease as determined by history, physical examination, electrocardiogram, urinalysis, and laboratory blood test results. Exclusion criteria included a fasting LDL-C level >190 mg/dL or triglyceride level >500 mg/dL and a fasting glucose >125 mg/dL. Subjects were excluded if they had an abnormal electrocardiogram, positive urinalysis, abnormal liver function tests, or a history of asthma, hypertension, or diabetes.

Design

The study was designed to determine tolerability and dosing level of the emulsion. The secondary goal was to determine the effect of emulsion infusion on serum lipids and lipoproteins. This was a double-blind, placebo-controlled crossover study at 3 different dose levels (75, 150, 300 mg/kg) of emulsion. The dosing was based on the amount of soy phospholipid in the emulsion. At each dose level, 10 male volunteers were admitted to the Rockefeller University General Clinical Research Unit and received either an infusion of placebo (Intralipid diluted 1:64) or emulsion. Volunteers were readmitted 1 week later to receive the other option.

At the low- and mid-dose levels, a single infusion over a 2-hour period was given. At the high-dose level of 300 mg/kg, the volunteers received the infusion over a 6-hour period divided into a 2-hour prime at 100 mg/kg/h and then 4 hours at 25 mg/kg/h. This dosing was chosen to achieve a phospholipid concentration of 500 mg/dL and simulate dosing for future studies using endotoxin administration in a human model of endotoxemia.

Subjects fasted, except for water, from 2200 hours the evening prior to receiving the emulsion/placebo infusion until 1000 hours the day after receiving the infusion (36 h total fasting). Blood was sampled for toxicity measures at screening, 0 hours, 6 hours, 24 hours, and 1 week after infusion. Levels of phospholipid, bile acids, and lipoproteins were sampled at screening, hours 0, 1, 2, 3, 4, 6, 12, and 24, and 1 week after infusion initiation.

Emulsion/placebo

The emulsion was manufactured by Fresenius Kabi.\textsuperscript{a} Soy phosphatidylcholine\textsuperscript{b} and soy triglyceride\textsuperscript{c} were mixed (92.5\% phosphatidylcholine: 7.5\% triglyceride) and emulsified in 18 mM sodium cholate, containing 2.6\% glycerol (w/v) by repeated passes through an APV Gaulin homogenizer at 10 000 PSI. The final mixture, containing 100 mg/mL phosphatidylcholine, was sterilized by passage through a 0.45-\textmu m membrane. The placebo was made from 20\% Intralipid diluted 1:64 in NaCl 0.9\%, thereby providing a solution that was slightly opaque but containing only trivial amounts of lipid. The emulsion and placebo were supplied in 100-mL glass bottles and were wrapped in aluminum foil to obscure any color difference between emulsion and placebo. Volunteers received a volume of emulsion/placebo of 0.75 mL/kg (low dose), 1.5 mL/kg (mid dose), and 3 mL/kg (high dose) based on the 3 dose levels and 100 mg/mL phosphatidylcholine concentration of the emulsion.

Adverse events

Major organ systems were monitored for potential toxicity by determining hepatic enzymes (lactic dehydrogenase [LDH], alkaline phosphatase, alanine aminotransferase [ALT], aspartate aminotransferase [AST]), kidney function parameters (blood urea nitrogen, creatinine, urinalysis), changes in hematologic (white blood cell count, platelet count, hemoglobin), hemolytic parameters (LDH, indirect bilirubin, haptoglobin, reticulocyte count), and prothrombin time at baseline, 6 hours, 24 hours, and 7 days following infusion. A 12-lead electrocardiogram was performed at baseline and 24-hour times. Adverse events were rated by severity (mild, moderate, severe) and relationship to study drug (not related, remote, possibly related, probably related). Safety monitors were also graded using the Common Toxicity Criteria (http://ctep.info.nih.gov/reporting/ctc.htm). Stopping criteria for the study were an ALT or AST value >5 times upper limits of normal or grade 3 or 4 toxicity.

Measurement of Apolipoproteins, Phospholipid, and Cholic Acid

Apolipoproteins A-I and B were measured using an immunoturbidimetric method (Roche Diagnostic Systems, Indianapol, IN).\textsuperscript{29} Phospholipid was measured using an enzymatic method based on measurement of the choline content of phosphatidylcholine, sphingomyelin, and lysophosphatidylcholine.\textsuperscript{30} Cholic acid was measured using an enzymatic test kit for total serum bile acids.\textsuperscript{31}

Pharmacokinetics

The peak concentrations of serum phospholipid and bile acid were taken as the maximum concentration at any point during the infusion day. To determine whether there was a linear relationship between dose and levels, the 2-hour time point was used in these calculations for all 3 dosing
groups. This was necessary due to the modification in the administration protocol for the high-dose group. The 24-hour AUC was calculated from the concentration–time curve by the trapezoidal rule. The serum half-life was estimated by fitting the incremental change in serum phospholipid (emulsion vs. placebo) over time to a monoexponential decay model.

STATISTICS

Data are presented as mean ± SD by dose group and time. Two-way ANOVA (with Dunnet’s correction for multiple comparisons) was used to test for significance of pairwise differences (treatment vs. placebo), with dose and time as factors.

Results

ADVERSE EVENTS

No clinically significant safety concerns were identified. There were no dropouts and all subjects successfully completed the protocol. There were 44 adverse events reported from 23 volunteers (Table 1); 27 events were noted in the emulsion period and 17 were noted in the placebo period. This difference was due to events unrelated or remotely related to the infusion. Of adverse events thought to be possibly or probably related, 5 occurred in the emulsion period and 4 developed in the placebo period. All of the adverse events were classified as mild and had a Common Toxicity Criteria grade of 1, except for a single grade 2 episode of nausea and vomiting that occurred during the placebo period. No adverse event required modification of the placebo or emulsion infusions. The most frequent complaints were gastrointestinal related (9 in the emulsion group; 7 in the placebo group). One volunteer had an asymptomatic elevation in his hepatic enzyme tests at the 1-week follow-up visit that was reported as an adverse event. He had a less than 2 times the upper limit of normal increase in ALT (80 U/L) and AST (65 U/L) 7 days after he received the emulsion infusion. Values returned to normal on retesting. This individual had fluctuations in liver-related enzymes that were noted during the placebo period as well as the emulsion period.

Four adverse events were related to the blood sampling or intravenous site in the emulsion period and none was noted during the placebo period. However, these events were not considered related to the emulsion since 3 of the 4 events were associated with blood sampling from the arm contralateral to the emulsion infusion.

LABORATORY SAFETY PANELS

There were no appreciable differences between placebo and emulsion treatments in hepatic enzyme, renal, hematologic, or coagulation tests. No volunteer exceeded the laboratory thresholds for stopping the study. There was an increase in bilirubin, predominantly indirect in origin, at the 24-hour time point for all dose levels for both placebo- and emulsion-treated groups. The increase in bilirubin was dose dependent only for the emulsion infusion and statistically different from placebo (p < 0.05) in the mid- and high-dose groups. At the 24-hour time point for the high-dose level, there was a mean 0.9 mg/dL greater increase in indirect bilirubin for the emulsion group (2.0 mg/dL emulsion vs. 1.1 mg/dL placebo). There were no associated differences in hepatic enzyme tests (LDH, alkaline phosphatase, ALT, or AST levels) between the control and emulsion groups at the 24-hour time point.

There was no evidence for overt hemolysis as an explanation for the increase in bilirubin. No significant differences were found between the emulsion and placebo groups for hemoglobin concentrations and reticulocyte counts. There was a slight reduction in haptoglobin level for the high-dose emulsion period that was significantly different (p < 0.05) from the placebo at the 6-hour time point (emulsion, 49 mg/dL vs. placebo, 64 mg/dL).

PHOSPHOLIPID CONCENTRATIONS

Figure 1 shows the clearance kinetics for serum phospholipid for all 3 dose groups compared with the averaged placebo groups. The shape of the curve for the high-dose group differed from that for the mid- and low-dose groups since the emulsion was given over 6 hours in the high-dose group. The maximum mean phospholipid concentration increased with dose, peaking at 709 ± 86 mg/dL. Significant differences between emulsion and placebo were observed at all time points, except for the 24-hour point in the low-dose group.

The AUC for phospholipids is shown in Figure 2. After the termination of the infusion, serum phospholipid concentrations dropped steadily to near baseline levels at 24 hours. Serum phospholipid half-life was 5.4 ± 0.6, 5.4 ± 0.5, and 8.0 ± 0.8 hours for the low-, mid- and high-dose emulsion infusions, respectively. The half-life for the high-dose group was significantly longer (p < 0.05) than the

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*There were 10 volunteers for each of the 3 dose levels. Adverse events were recorded and tabulated during each treatment phase prior to unblinding of the treatment assignment.*
half-life for the low- and mid-dose groups. Linear dose–response relationships were observed between dose and peak concentration and dose and AUC (Figure 2). A plateau phospholipid concentration was not reached in the high-dose group, since the time of infusion of the emulsion was only 6 hours compared with the phospholipid half-life of approximately 8 hours.

**Bile Acid Concentrations**

Figure 1 shows the clearance kinetics for serum bile acid for the treatment and placebo groups. The maximum bile acid concentrations occurred at the end of the 2-hour priming dose for all 3 groups in contrast to phospholipid levels, which peaked in the high-dose group at the end of the 6-hour infusion. Serum half-life for bile acid (low dose, 38.7 ± 12; mid, 26.8 ± 8; high, 88.7 ± 31 min) was much shorter than that of phospholipid. The half-life at the high dose was longer (p > 0.05) than for the low dose.

**Cholesterol, Triglyceride, and Apolipoprotein Levels**

The infusion of a phospholipid-rich emulsion was associated with a dose- and time-dependent upward trend in the total cholesterol level (Figure 3). The increase in total cholesterol level was primarily due to an increase in LDL-C. Triglyceride levels also increased, since the emulsion contains 7.5% of its lipid content as triglyceride.

HDL-C in the high-dose group decreased immediately following termination of the emulsion infusion, but rebounded to above placebo levels by the 24-hour time point (p = 0.01). In contrast, apolipoprotein A-I, the primary apolipoprotein of HDL-C, increased steadily during the course of the emulsion infusion, indicating less cholesterol per HDL particle (Figure 4). LDL-C behaved differently from HDL-C in that the cholesterol content of LDL relative to apolipoprotein B was found to increase during the emulsion infusion period (Figure 4).

**Discussion**

The goal of this investigation was to determine the safety and pharmacokinetics of a novel phospholipid-rich emulsion. The intent was to obtain phospholipid levels in human volunteers equivalent to or above levels found to be protective in a porcine sepsis model (300–500 mg/dL). Peak phospholipid levels of 700 mg/dL were achieved in the human high-dose group without dose-limiting adverse reactions.

A natural human model for elevated phospholipid levels are patients with primary biliary cirrhosis. These patients may present with serum phospholipid concentrations >1000 mg/dL. These lipid levels are associated with red blood cell abnormalities and a mild compensated hemolytic anemia. Not surprisingly, the most significant adverse effect found in preclinical studies performed in pigs was hemolysis, most prominent when the emulsion was given at doses that achieved phospholipid levels >2000 mg/dL. There was no evidence for overt hemolysis in human volunteers at doses achieving peak phospholipid levels of 700 mg/dL as supported by the lack of significant differences in LDH, hematocrit, hemoglobin, or reticulocyte counts between the placebo and emulsion groups. However, the dose-dependent increase in indirect bilirubin and slight decrease in haptoglobin at the 6-hour time point in the high-dose emulsion group may have been due to enhanced red blood cell removal from the circulation.

There were 2 distinct observations related to the rise in indirect bilirubin. First, an increase in the indirect bilirubin level in the placebo...
groups occurred, which was maximal at the 24-hour time point when volunteers had been fasting for approximately 36 hours. This increase is a well-described phenomenon and is related to fasting for >24 hours. In addition, there was a dose-dependent increase in indirect bilirubin in the emulsion-treated groups. The greatest increase in indirect bilirubin was found in volunteers with the highest baseline indirect bilirubin levels.

The lipid emulsion redistributes its components onto lipoproteins in the plasma compartment. Previous studies in pigs have shown that the emulsion phospholipid associates with preexisting lipoproteins and can also form...

Figure 2. Dose–response: Maximum serum concentrations and AUC. Data are pairwise mean changes and standard deviations. Left panels show the maximum serum concentration of phospholipid (PL) (top) and bile acids (BA) (bottom). Right panels show the AUC of phospholipid (top) and bile acids (bottom). The time of maximum concentration was taken at the end of the 2-hour priming dose for the purpose of testing for a linear dose–response relationship. Note that the high-dose group received a prime of 200 mg/kg over 2 hours, followed by an infusion of 25 mg/kg for 4 hours. The AUC was calculated over 24 hours.

Figure 3. Serum lipid and lipoprotein concentrations by dose and time. Data are group mean changes in serum lipids calculated as the difference between treatment and placebo for each patient. Pairwise differences (emulsion vs. placebo) in total cholesterol are significant in the mid- and high-dose groups from 2 through 8 hours. Pairwise differences in triglycerides are significant only in the high-dose group from 4 through 12 hours. Pairwise differences in low-density lipoprotein cholesterol (LDL-C) are significant in the mid-dose group from 2 through 12 hours and from 2 through 24 hours in the high-dose group. Pairwise differences in high-density lipoprotein cholesterol (HDL-C) are significant in all dose groups from 4 through 12 hours.
lipoprotein-x–like phospholipid vesicles. However, the pigs in that study were septic and did not show the increase in serum cholesterol that we observed in the current study. In healthy human volunteers, the increase in serum cholesterol occurred predominantly in the LDL fraction. The most likely source is efflux from tissue, although decreased lipoprotein catabolism or increased cholesterol synthesis cannot be ruled out. The lack of increase in apolipoprotein B100 concentration suggests that decreased LDL uptake by the LDL receptor is not the major cause for the increase in cholesterol concentration. The transient decrease in HDL-C while apolipoprotein A-I is steady or increasing is indicative of a shift to phospholipid-rich, cholesterol-poor HDL particles. This effect may be explained by selective clearance of HDL phospholipid and cholesterol into the liver via hepatic scavenger receptor B-I and excretion into bile. This selective removal causes HDL lipid to turn over 4–6 times faster than HDL protein (apolipoprotein A-I). Coadministration of cholic acid with HDL was found to additionally increase hepatic uptake and biliary secretion of HDL phospholipid.

There are several limitations of this study. Since the half-life of the emulsion is related to the half-life of the lipoprotein particles that incorporate the phospholipid, additional studies that include lipoprotein remodeling would help determine why the half-life of the phospholipid was longer in the high-dose group. Studies of the emulsion in critically ill patients will also be necessary. It is likely that sepsis and the acute phase will induce changes in metabolism that will significantly impact on the pharmacokinetics of the emulsion. Whether the emulsion will have a shorter or longer half-life in critically ill patients is unclear. Phospholipid levels are decreased in acutely ill subjects, possibly due to an increase in lipid catabolism. However, it is also possible that decreased liver function in sepsis would slow the catabolism of the emulsion.

**Summary**

Sepsis has a significant incidence of mortality for which there are currently limited therapeutic options. Infusion of a unique lipid emulsion achieved serum concentrations of phospholipid that have been shown in animal models to neutralize endotoxin, one of the primary initiators of septic shock. Based on the favorable safety profile and pharmacokinetic data, studies of this phospholipid-rich emulsion in subjects with endotoxemia are warranted.

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**Figure 4.** Changes in apolipoproteins and the ratio of cholesterol to apolipoprotein concentrations. Data are shown for the high-dose emulsion group. Significant differences (p < 0.05) between emulsion and placebo are indicated by *. apo = apolipoprotein; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
References


horas para la dosis baja, media y alta. Aumentos en los niveles de colesterol total, colesterol LDL, y apolipoproteínas A-I y B fueron observados. El colesterol HDL disminuyó inmediatamente después de la infusión de la emulsión, pero rebotó a niveles sobre los del placebo en 24 horas.

CONCLUSIONES: Una emulsión única rica en fosfolípidos demostró tener un perfil de seguridad favorable y expandió la fuente de lipidos sanguíneos y lipoproteínas sin usar productos derivados de sangre humana. Se alcanzaron niveles lipídicos que se espera protejan contra los efectos fisiológicos de las endotoxinas bacterianas.

Sonia I Lugo

RÉSUMÉ

INTRODUCTION: Il a été démontré dans le modèle animal de septicémie que les lipides et les lipoprotéines, en se fixant à l’endotoxine, peuvent neutraliser les effets physiologiques de celle-ci et ainsi améliorer la condition des animaux.

OBJECTIF: Étudier l’innocuité et la pharmacocinétique d’une émulsion riche en phospholipides et exempte de protéines humaines développée pour neutraliser l’endotoxine. Étudier les effets de cette émulsion sur les lipides et lipoprotéines séricques.

MÉTHODOLOGIE: Trente hommes sains (entre 18 et 40 ans) ont reçu une émulsion contenant 92.5% de phospholipides de soya, 7.5% de triglycérides de soya, et 18 mM de cholate de sodium dans le cadre d’une étude en chassé-croisé à répartition aléatoire et à double insu. Un placebo et 3 doses croissantes (75, 150, 300 mg/kg) de phospholipides ont été administrés par perfusion intraveineuse pendant 2 heures pour les doses de 75 et 150 mg/kg et pendant 6 heures pour la dose de 300 mg/kg.

RÉSULTATS: Aucune toxicité significative n’a été observée. Une légère augmentation de la bilirubine indirecte, en relation avec la dose, a été observée à 24 heures. L’écart maximal entre le placebo et l’émulsion était de 0.9 mg/dL. Les concentrations maximales de phospholipides étaient de 316 ± 30; 533 ± 53, et 709 ± 86 mg/dL alors que les demi-vies d’élimination étaient de 5.4 ± 0.6, 5.4 ± 0.5, et 8.0 ± 0.8 heures, respectivement, pour les 3 différentes doses. Des augmentations des niveaux de cholestérol total, de cholestérol LDL, et des apoprotéines A-I et B ont été observées. Les niveaux de cholestérol HDL ont diminué immédiatement après l’administration de la perfusion mais ont remonté à des niveaux supérieurs au placebo après 24 heures.

CONCLUSIONS: L’émulsion étudiée, riche en phospholipides, a un profil d’innocuité acceptable qui permet d’augmenter les lipides et lipoprotéines du sang sans avoir recours à des dérivés du sang humain. Les niveaux de lipides atteints sont dans les valeurs supposées protéger contre les effets physiologiques de l’endotoxine.

Suzanne Laplante