MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION EXACERBATES PERITONITIS-INDUCED SEPTIC SHOCK IN RATS: ROLE ON CELL MEMBRANE REMODELING

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ABSTRACT—Background and aims: Lipid emulsions for parenteral nutrition interfere with immunity and may alter the cell plasma membrane and microparticle release, thus modulating their biological effects. Our aim was to evaluate the effect of two lipid emulsions for parenteral nutrition containing either a mixture of long- and medium-chain triglycerides (LCTs and MCTs) or LCTs only, to assess their role on microparticle release and acute inflammation during septic shock in rats. **Methods and results:** Septic rats (cecal ligation and puncture) and sham rats were infused with 5% dextrose or a lipid emulsion during 22 h. After 18 h, rats were resuscitated during 4 h and hemodynamic parameters monitored. Circulating microparticles and their phenotype were measured by prothrombinase assay; heart and aorta were collected for Western blotting and electron paramagnetic resonance measurements. No significant effect of lipid emulsions was observed in sham rats. In septic rats, norepinephrine requirements were increased in MCT/LCT-infused rats compared with 5% dextrose- or LCT-infused rats (2.7 ± 0.2 vs. 1.9 ± 0.8 and 1.2 ± 0.3 $\mu\text{g/kg}$ per minute, respectively; $P < 0.05$) with increased procoagulant microparticle generation (38.6 ± 5.8 vs. 18.8 ± 3.1 and 19.2 ± 3.0 nM equivalent phosphatidylserine [Eq PhtdSer]; $P < 0.05$), leukocyte- (17.4 ± 3.5 vs. 7.7 ± 1.8 and 6.0 ± 1.1 nM Eq PhtdSer; $P < 0.05$), platelet- (13.9 ± 2.5 vs. 4.4 ± 0.7 and 5.4 ± 1.3 nM Eq PhtdSer; $P < 0.05$), and endothelial-derived microparticles (16.9 ± 3.6 vs. 6.4 ± 1.4 and 5.6 ± 0.8 nM Eq PhtdSer; $P < 0.05$). The mixture of MCTs/LCTs significantly increased cardiac and vascular nitric oxide and superoxide anion production, phosphorylated I κ B, and cyclooxygenase 2 expression compared with the lipid emulsion containing only LCTs. **Conclusions:** Compared with 5% dextrose, MCT/LCT supplementation during septic shock in rats induced deleterious effects with increased inflammation and cell activation, associated to vascular hyporeactivity. During septic shock, LCT supplementation seemed to be neutral compared with 5% dextrose infusion.

KEYWORDS—Lipid emulsions, parenteral nutrition, membrane remodeling, inflammation, microparticles, septic shock

INTRODUCTION

Fatty acids (FAs) are known to interfere with many physiological processes, including inflammation and immunity (1). Inflammatory response modulation in critically ill patients through the modulation of lipid content in parenteral emulsion has therefore aroused clinician interest (2). The composition of lipid emulsions may account for changes in cell membrane composition, downstream production of eicosanoids and cytokines, and gene expression (1, 3). Exogenous lipids could alter the cell membrane fluidity and the lipid raft organization, thereby interfering with the activity of key membrane proteins involved in immune cell signaling (4). Several experimental

studies have evidenced the regulatory role of lipid rafts after incorporation of n-3 polyunsaturated FAs (PUFAs) that limited T-cell activation and the immune response (5–7). The n-6 and n-3 PUFA content of immune cell plasma membranes can be modulated by eicosapentaenoic and docosahexaenoic acid intake (3). Interestingly, n-6 PUFA ingestion may acutely alter the endothelial function, resulting in an increased generation of circulating endothelial microparticles (MPs) (8). Microparticles are plasma membrane submicron vesicles, released from the cell in response to inflammatory signals following the translocation of procoagulant phosphatidylserine (PhtdSer) in the outer leaflet (9). Numerous experimental studies have also demonstrated that MPs behave as vascular and cellular effectors (10, 11). In sepsis, circulating MPs may indeed promote inflammation and vascular cell apoptosis and contribute to hemodynamic dysfunction, coagulation activation, and multiple organ failure (10, 12). The purpose of this study was therefore to assess the effects of two parenteral nutrition lipid emulsions on the modulation of inflammation, procoagulant MP release, and hemodynamic during septic shock in rats.

MATERIALS AND METHODS

This study was performed with the approval of the Strasbourg Regional Committee of Ethics in Animal Experimentation (CREMEAS, AL/69/76/02/13).

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Industrial Lipid Emulsions for Parenteral Nutrition

Intralipid (soybean oil 200 g/L—long-chain triglycerides [LCTs]) was from Fresenius-Kabi (Bad Homburg, Germany) and Medialipid (soybean 100 g/L—LCTs, coconut oil 100 g/L—medium-chain triglycerides [MCTs]) from B Braun (Melsungen, Germany). Their n-3 PUFA content was only α -linolenic acid (18:1 n-3) and neither of the emulsions contained eicosapentaenoic acid (20:5 n-3) or docosahexaenoic acid (22:6n-3); the n-3/n-6 ratio was the same in both emulsions (see Table 1, Supplemental Digital Content 1, at <http://links.lww.com/SHK/A232>).

Cecal ligation and puncture model and infusion with lipid emulsions for parenteral nutrition

Male Wistar rats (300–350 g) were from Javier Labs (Le Genest-Saint-Isle, France). During surgical procedures, rats were anesthetized with isoflurane 1% to 2% (Baxter S.A.S., Maurepas, France), and analgesia was ensured by sufentanil (Mylan, Pittsburgh, Pa) (0.1 μ g/kg of body weight) administered subcutaneously. Before skin incision, a subcutaneous injection of 0.1 mL lidocaine 1% (AstraZeneca, Rueil-Malmaison, France) was performed (Table 1).

Rats underwent cecal ligation and puncture (CLP) as previously described (13). The rats developed septic shock within the 16 to 20 h after CLP. Sham rats underwent laparotomy and cecal exposure. The right internal jugular was catheterized, and the catheter was tunneled between the ears.

Rats were then allocated to six groups of 12 animals each: sham-G5 (dextrose 5%), sham-IL (Intralipid), sham-ML (Medialipid), CLP-G5, CLP-IL, and CLP-ML. The randomly allocated lipid emulsion was infused at identical rates during 22 h (1.5 g/kg per 24 h, according to human nutrition guidelines [14]). The control groups (sham-G5 and CLP-G5) were infused with 5% dextrose under identical conditions. The lipid emulsion or dextrose infusion was not stopped for the next 22 h until the end of the experiment. No animal died before the end of the experiment.

After 18 h, when CLP rats fulfilled illness criteria (lethargy, piloerection, glassy eyes), rats were anesthetized, tracheotomized, and mechanically ventilated. The right carotid artery was gently exposed, and a transit-time ultrasound flow probe of 2.0 mm (Transonic Systems, Ithaca, NY) was attached to the carotid artery to continuously measure carotid blood flow (CBF). The left femoral artery was used to measure mean arterial pressure (MAP) and heart rate (HR) and to collect blood samples. For the CLP group, septic shock was assessed if MAP was less than 90 mmHg and lactate was elevated.

In CLP groups, fluid resuscitation was performed with a bolus of 0.9% NaCl (500 μ L/10 min if MAP was <100 mmHg); if MAP remained less than 100 mmHg, norepinephrine was infused and increased by step of 0.1 μ g/kg per minute each 10 min to target a MAP greater than 100 mmHg.

After 240 min, rats were bled via the left carotid artery under sterile condition using endotoxin-free material. The total blood volume was collected in sodium citrate tubes (Vacutainer; Becton Dickinson; Le Pont de Claix, France); organs were collected for Western blotting analysis and electron paramagnetic resonance (EPR) measurements.

Quantification of procoagulant MPs

Blood samples were centrifuged for 15 min at 800g; plasma was centrifuged 2 min at 12,000g to obtain platelet-poor plasma. Platelet-poor plasma was centrifuged twice at 12,000g for 60 min to pellet MPs. The MP pellets were suspended in 150 μ L of 0.9% NaCl, pooled, and concentrated by centrifugation for 60 min at 12,000g. Washed pellet suspensions (500 μ L of 0.9% NaCl) were kept at 4°C for less than 2 weeks.

To specifically analyze the generation of procoagulant MPs, MP quantification was performed by functional prothrombinase assay as described elsewhere (15). Briefly, after three washes, the amount of MPs insolubilized onto annexin-5-coated microplates was measured by prothrombinase assay in which PhtdSer is the limiting factor of the reaction leading to thrombin generation. The measurements were performed in a kinetic mode with a thermostated spectrophotometer (VersaMax Molecular Device, Conquer Scientific, San Diego, Calif). The absorbance was converted into nanomolar equivalent PhtdSer (Eq PhtdSer) by reference to a calibration curve made of known concentrations of synthetic vesicles containing 33% wt/wt PhtdSer and 67% wt/wt phosphatidylcholine (15).

Characterization of the MP cell origin

Microparticle phenotype was assessed according to the expression of cellular specific membrane antigens using biotinylated monoclonal antibodies (anti-CD61: platelet GP_{IIb/IIIa} [β_3], anti-CD45: leukocyte common antigen, anti-CD54: intercellular adhesion molecule 1 for endothelial cells [BioLegend, San Diego, Calif], and antiglycophorin for erythrocytes [Becton Dickinson, Franklin Lakes, NJ]). Microparticles were measured after capture onto each specific antibody coated on the streptavidin plate (1 μ g/well) as described above.

Electron paramagnetic resonance

Nitric oxide—Aorta and heart samples were incubated for 30 min in Krebs-HEPES buffer containing: bovine serum albumin (20.5 g/L), CaCl₂ (3 mM), and L-arginine (0.8 mM). NaDETC (3.6 mg) (diethyldithiocarbamate [DETC]) and FeSO₄·7H₂O (2.25 mg) were separately dissolved under nitrogen gas bubbling in 10-mL volumes of ice-cold Krebs-HEPES buffer. These compounds were rapidly mixed to obtain a pale yellow-brown opalescent colloid Fe(DETC)₂ solution (0.4 mM), which was used immediately. The colloid Fe(DETC)₂ solution was added to the organs and incubated for 45 min at 37°C. Nitric oxide

TABLE 1. Characteristics of rats

	Sham-G5	Sham-IL	Sham-ML	CLP-G5	CLP-IL	CLP-ML
Weight, g	358 $\pm$ 9	358 $\pm$ 9	363 $\pm$ 5	349 $\pm$ 8	353 $\pm$ 7	353 $\pm$ 7
Diuresis, mL	14.9 $\pm$ 3.8	18.5 $\pm$ 2.4	17.6 $\pm$ 5.7	7.3 $\pm$ 2.3*	9.9 $\pm$ 2.4*	9.0 $\pm$ 2.4*
MAP, mmHg						
Baseline	119 $\pm$ 4	120 $\pm$ 3	120 $\pm$ 3	72 $\pm$ 6*	71 $\pm$ 7*	72 $\pm$ 6*
End experiment	116 $\pm$ 4	114 $\pm$ 7	117 $\pm$ 4	111 $\pm$ 7	106 $\pm$ 8	105 $\pm$ 7
HR, beats/min						
Baseline	356 $\pm$ 12	349 $\pm$ 19	358 $\pm$ 14	398 $\pm$ 31*	375 $\pm$ 12*	405 $\pm$ 16*
End experiment	381 $\pm$ 14	369 $\pm$ 20	391 $\pm$ 19	417 $\pm$ 40	390 $\pm$ 11	382 $\pm$ 29
CBF, mL/min						
Baseline	4.5 $\pm$ 0.8	4.0 $\pm$ 0.4	4.8 $\pm$ 0.3	2.6 $\pm$ 0.7*	2.9 $\pm$ 0.8*	2.8 $\pm$ 0.8*
End experiment	3.6 $\pm$ 0.5	3.2 $\pm$ 0.5	4.2 $\pm$ 0.6	2.5 $\pm$ 0.4*	2.7 $\pm$ 0.6	2.9 $\pm$ 0.6*
NaCl, mL/h	1.0 $\pm$ 0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	2.5 $\pm$ 0.5*	2.4 $\pm$ 0.3*	2.7 $\pm$ 1.1*
NE, μ g/kg per minute	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.9 $\pm$ 0.8*	1.2 $\pm$ 0.3*	2.7 $\pm$ 0.2*†
Lactate, mmol/L	1.1 $\pm$ 0.2	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	2.8 $\pm$ 0.4*	2.6 $\pm$ 0.1*	2.4 $\pm$ 0.2*
Survival, min	240 $\pm$ 0	240 $\pm$ 0	240 $\pm$ 0	240 $\pm$ 0	240 $\pm$ 0	240 $\pm$ 0

Values are presented as mean (SD).

For each group, n = 12. * P < 0.05 vs. sham-G5, † P < 0.05 vs. CLP-IL.

NE indicates norepinephrine; baseline, 18h after CLP/sham operation; end of experiment, 22h after CLP/sham operation, MPs, microparticles; nM Eq PhtdSer, nanomolar PhtdSer equivalents.

FIG. 1. **Quantification (A) and phenotype (B) of microparticles.** $n = 12$, * $P < 0.05$ vs. CLP-IL, # $P < 0.05$ vs. sham-G5, and & $P < 0.05$ vs. CLP-G5.

(NO•) measurement was performed on a table-top x-band spectrometer Miniscope (MS200; Magnettech, Berlin, Germany). Recordings were made at 77°K, using a Dewar flask. Instrument settings were 10 mW of microwave power, 1 mT of amplitude modulation, 100 kHz of modulation frequency, 60 s of sweep time, and five numbers of scans.

Superoxide anion—Aorta and heart samples were allowed to equilibrate in deferoxamine-chelated Krebs-HEPES solution containing 1-hydroxy-3-methoxycarbonyl 2,2,5,5-tetramethylpyrrolidin (CMH; Noxygen, Elzach, Germany) (500 μ M), deferoxamine (25 μ M), and DETC (5 μ M) under constant temperature (37°C) for 1 h. The reaction was stopped by freezing the samples in liquid nitrogen for EPR spectroscopy analysis.

Values were expressed as arbitrary units per milligram weight of dried tissue (A/Wd).

Western immunoblotting

Phosphorylated I κ B- α (pI κ B- α), nuclear factor κ B (NF- κ B), and cyclooxygenase 2 (COX-2) expression was evaluated in heart and aorta lysates using Western immunoblotting. Proteins were separated by electrophoresis in 10% polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane. Nonspecific binding sites were blocked with 5% skimmed milk in Tris-buffered saline solution with 0.5% Tween for 1 h at room temperature. Membranes were then incubated overnight at 4°C with primary antibodies directed against pI κ B- α (1/500; Euromedex, Souffelweyersheim, France), NF- κ B p65 (1/1,000; Ozyme, Saint Quentin, France), COX-2 (1/1,000; Cayman Chemical Co, Ann Arbor, Mich), and β -tubulin (1/10,000; Santa Cruz Biotechnology, Heidelberg, Germany). After three washes, membranes were incubated with secondary antibodies (1/5,000 dilution except for anti- β -tubulin that revealed with a 1/40,000 dilution of anti-mouse immunoglobulin G) for 1 h at room temperature, and signals were revealed by chemiluminescence. The optical density was quantified and expressed relative to β -tubulin (ImageJ, National Institutes of Health, Bethesda, Md).

Statistical Analysis

The intergroup comparison of hemodynamic parameters was performed by analysis of variance repeated measures; the pairwise comparison was made using a Tukey-Kramer adjustment for P values. A nonparametric analysis by Kruskal-Wallis test with Dunn post hoc test was used for analysis of MPs, proteins, NO•, and superoxide anion ($O_2^{\bullet -}$). All statistics was performed with

Statview software (version 5.0; SAS Institute, Cary, NC). All values are presented as mean $\pm$ SD for n experiments, with n representing the number of rats. $P < 0.05$ was considered statistically significant.

RESULTS

The emulsion containing a mixture of MCTs and LCTs increases septic shock-induced vascular hyporeactivity

Lipid emulsions had no adverse effect in sham rats (sham-IL and sham-ML), as hemodynamic parameters (HR, MAP, CBF), MPs, Western immunoblotting, and EPR were unaltered compared with 5% dextrose-infused sham rats (sham-G5). The infusion of lipid emulsions containing LCTs (IL) or a mixture of LCTs/MCTs (ML) had no effect (MAP, HR, CBF, and blood lactate) compared with 5% dextrose infusion on the early phase of infection in CLP rats, up to the 18th hour after surgery (see Table I, Supplemental Digital Content 1, at <http://links.lww.com/SHK/A232>).

At the end of the 4-h resuscitation period (18th to 22th hour), MAP, HR, CBF, and blood lactate were still not significantly different, and all CLP rats survived (see Table I, Supplemental Digital Content 1, at <http://links.lww.com/SHK/A232>). Nevertheless, the LCT/MCT emulsion increased septic shock-induced vascular hyporeactivity evidenced by an increased norepinephrine requirement to reach the targeted MAP compared with 5% dextrose (2.7 ± 0.2 vs. 1.9 ± 0.8 μ g/kg per minute; $P < 0.05$), whereas LCT emulsion had no effect (1.2 ± 0.3 vs. 1.9 ± 0.8 μ g/kg per minute, not statistically significant) (see Table I, Supplemental Digital Content 1, at <http://links.lww.com/SHK/A232>).

FIG. 2. **Vascular and cardiac nitrosative and oxidative stresses.** Nitric oxide and $O_2^{\bullet -}$ measured by EPR in the aorta (A and C) and heart (B and D). $n = 6$, * $P < 0.05$ vs. CLP-IL, # $P < 0.05$ vs. sham-G5, & $P < 0.05$ vs. CLP-G5.

FIG. 3. Vascular and cardiac pIkB- α and COX-2 expressions relative to β -tubulin content evaluated by Western immunoblotting in the aorta (A and C) and heart (B and D). $n = 6$, * $P < 0.05$ vs. CLP-IL, # $P < 0.05$ vs. sham-G5, & $P < 0.05$ vs. CLP-G5.

The emulsion containing a mixture of MCTs and LCTs alters membrane vesiculation

In the CLP groups (CLP-G5, CLP-IL, CLP-ML), total MPs and leukocyte-, platelet- and endothelial-derived MPs were significantly increased compared with sham rats (Fig. 1, A and B). Microparticle generation was significantly increased in CLP-ML rats compared with CLP-G5 rats and CLP-IL rats (38.6 ± 5.8 vs. 18.8 ± 3.0 and 19.2 ± 3.0 nM Eq PhtdSer, respectively; $P < 0.05$) (Fig. 1A), as well as leukocyte- (17.4 ± 3.5 vs. 7.7 ± 1.8 and 6.0 ± 1.1 nM Eq PhtdSer, respectively; $P < 0.05$), platelet- (13.9 ± 2.5 vs. 4.4 ± 0.7 and 5.4 ± 1.3 nM Eq PhtdSer, respectively; $P < 0.05$), and endothelial-derived MPs (16.9 ± 3.6 vs. 6.4 ± 1.4 and 5.6 ± 0.8 nM Eq PhtdSer, respectively; $P < 0.05$) (Fig. 1B). Of note, MP generation (concentration and phenotype) was not significantly different between CLP-IL and CLP-G5 rats (Fig. 1, A and B).

The emulsion containing a mixture of MCTs and LCTs increases cardiovascular oxidative and nitrosative stresses

Nitric oxide and $O_2^{\bullet -}$ productions were significantly increased in the aorta (174.8 ± 14.7 vs. 130.2 ± 18.5 and 708.2 ± 35.6 vs. 496.3 ± 45.8 amplitude/Wd, respectively; $P < 0.05$) (Fig. 2, A and C) and heart (158.7 ± 15.7 vs. 110.3 ± 7.3 and 786.3 ± 29.6 vs. 579.1 ± 62.6 amplitude/Wd, respectively; $P < 0.05$) of CLP-ML rats compared with CLP-IL rats (Fig. 2, B and D). Compared with sham rats, $NO^{\bullet}$ and $O_2^{\bullet -}$ were significantly increased in aorta and heart from CLP-G5 rats but not in aorta and heart from CLP-IL rats (Fig. 2, A–D). Nitric oxide and $O_2^{\bullet -}$ productions were not different in CLP-IL and CLP-G5 groups, except a significant increase in cardiac $NO^{\bullet}$ in CLP-IL rats compared with CLP-G5 rats (Fig. 2B).

The emulsion containing a mixture of MCTs and LCTs modulates vascular inflammation

Nuclear factor κB activation was evidenced through the assessment of the phosphorylation of its inhibitor I κB , resulting in the translocation of free NF- κB to the nucleus and the transcription of proinflammatory genes. In the aorta, pIkB- α was significantly increased in CLP-G5 rats compared with sham-G5 rats (0.2 ± 0.0 vs. 0.1 ± 0.0 relative to β -tubulin content; $P < 0.05$) as well as in CLP-ML rats compared with CLP-IL rats (0.4 ± 0.1 vs. 0.1 ± 0.0 relative to β -tubulin content; $P < 0.05$) (Fig. 3A), whereas NF- κB expression remained

unchanged (data not shown). Similarly, in heart samples of CLP-G5 rats, pIkB- α expression was significantly increased compared with sham-G5 rats (1.0 ± 0.2 vs. 0.3 ± 0.1 relative to β -tubulin content; $P < 0.05$), whereas NF- κB expression remained unaltered (data not shown). Moreover, cardiac pIkB- α expression was significantly increased in CLP-ML rats compared with CLP-IL rats (1.8 ± 0.1 vs. 0.6 ± 0.2 relative to β -tubulin content; $P < 0.05$) (Fig. 3B). Finally, pIkB- α was significantly increased in CLP-ML group compared with CLP-G5 in both heart and aorta (Fig. 3, A and B).

In the aorta, COX-2 expression was significantly increased in CLP-G5 rats compared with sham-G5 rats (1.4 ± 0.2 vs. 0.9 ± 0.2 relative to β -tubulin content; $P < 0.05$), as well as in CLP-ML rats compared with CLP-IL (2.1 ± 0.3 vs. 1.0 ± 0.1 relative to β -tubulin content; $P < 0.05$) (Fig. 3C). Cardiac COX-2 expression was also significantly increased in CLP-G5 rats compared with sham-G5 rats (1.4 ± 0.1 vs. 0.4 ± 0.0 relative to β -tubulin content; $P < 0.05$). Cyclooxygenase 2 expression was also significantly increased in CLP-ML rats compared with CLP-IL rats (1.5 ± 0.2 vs. 0.5 ± 0.0 relative to β -tubulin content; $P < 0.05$) (Fig. 3D). Cyclooxygenase 2 was significantly increased in CLP-ML group compared with CLP-G5 in both heart and aorta (Fig. 3, C and D).

DISCUSSION

Fatty acid supplementation leads to plasma membrane remodeling and may thus affect immune functions with clinical consequences in patients with inflammatory diseases (1). We therefore hypothesized that lipid emulsion supplementation could alter membrane vesiculation and MP release, thereby modulating inflammation and hemodynamic status in septic rats (3, 10, 12, 16). Thus, we investigated the hemodynamic function of septic rats and used MPs as surrogates of vascular cell damage.

In the present study, we have not shown any beneficial or deleterious effects of LCT or LCT/MCT parenteral lipid emulsions on sham rats. Moreover, these emulsions did not prevent or worsen septic shock issue. Nevertheless, we have shown that the infusion of a mixture of MCTs and LCTs may exacerbate the septic shock-induced hemodynamic dysfunction, enhancing the activation of vascular cells, inflammatory processes, and cardiovascular oxidative and nitrosative stresses.

We have also shown that the emulsion containing a mixture of MCTs and LCTs (ML) significantly increased membrane vesiculation in septic rats, compared with the emulsion containing only LCTs (IL) or with 5% dextrose. These data are consistent with previous works showing that FAs are differentially incorporated into the cell membrane depending on the length of their carbon chain, which leads to increased membrane vesiculation with increased phospholipid membrane disorder in emitted MPs (17, 18). In contrast, n-3 FAs decrease the concentration of circulating MPs in post-myocardial infarction patients, suggesting that n-3 FAs might alter membrane vesiculation (19). In addition, recent data from our laboratory suggest that a mixture of MCTs and LCTs would have an energetic function, but would also take part in membrane vesiculation, by being incorporated in membranes and by altering their physical order (18).

To further characterize the *in vivo* FA effects on membrane vesiculation, we have established the phenotype of circulating MPs. We focused on procoagulant MPs exposing PhtdSer, which have been shown to behave as pathogenic markers in experimental septic shock and as markers of coagulation disturbance in clinical trials (10, 20). We have thus shown that a mixture of MCTs and LCTs significantly alters the MP phenotype, with an increased generation of circulating leukocyte-, platelet-, and endothelial-derived MPs, reflecting their parental cell activation. In contrast, LCTs do not seem to have any effect on cellular activation in septic rats as indicated by the unchanged MP pattern.

Furthermore, lipid emulsions containing a mixture of MCTs and LCTs promoted enhanced proinflammatory markers in aorta and heart tissues. Therefore, MCT infusion could lead to procoagulant and proinflammatory MP release, which might in turn deeply alter the cell membrane and increase further cell damages.

Lipid emulsions containing a mixture of MCTs and LCTs would have specific clinical advantages over lipid emulsions containing LCTs alone. Several studies indeed suggest their superiority, with improved nutritional status in septic and cancer patients undergoing surgery (21, 22). Indeed, the MCT/LCT emulsion was shown to increase patient prealbumin levels, which would be partially due to the higher circulating insulin levels. However, the outcome of septic patients or the length of hospital stay, the surgical complication rate in tract cancer patients following surgery was not significantly different, whatever the emulsion considered. Moreover, a recent review by Casaer and van den Berghe (23) failed to evidence any benefit in lipid modulation during septic shock.

More interestingly, emulsions containing a mixture of MCTs and LCTs would be less immunosuppressive than LCT emulsions. The former would be associated with reduced alterations of human monocyte and neutrophil functions (24), with subsequent limited infections in patients with chronic obstructive pulmonary disease (25). Experimental and clinical data, however, diverge, as in patients with acute respiratory distress syndrome, Masclans et al. (26) showed that LCT emulsions increased cardiac output and oxygen delivery, whereas Smyrniotis et al. (27) showed that it worsened oxygenation.

Fatty acids may interfere with inflammation by numerous mechanisms, including their incorporation into the phospholipids of inflammatory cell membranes, as we have evidenced

it in monocytes after LPS stimulation (18) and as it has been shown in other models (5). Furthermore, FAs are known to affect inflammation through the NF- κ B pathway. Indeed, n-3 FAs inhibit the LPS-induced production of inflammatory proteins such as COX-2, *in vitro* and *in vivo* (3). In our study, a mixture of MCTs and LCTs would have the opposite effect and upregulate the NF- κ B pathway with increased I κ B phosphorylation and subsequent inflammatory protein expression, as it has been shown *in vitro* with saturated FAs, especially lauric acid (C12:0) (28, 29). In contrast, LCTs seemed neutral compared with 5% dextrose.

Some FA receptors may be involved in the regulation of these anti-inflammatory effects such as the peroxisome proliferator-activated receptor, a transcription factor that downregulates the NF- κ B pathway when bound to PUFAs (30). The LCT receptor GPR120, which is highly expressed on inflammatory cells mediating anti-inflammatory gene activation (31), and the MCT proinflammatory receptor GPR84, which promotes cytokine secretion in LPS-treated neutrophils and macrophages (32), could also have contributed to the respective effects of MCTs and LCTs in our study. Moreover, several LCTs have been shown to decrease leukocyte chemotaxis toward inflammatory sites, contrary to MCTs (32, 33). N-3 LCTs also decrease arachidonic acid-derived proinflammatory eicosanoids and COX-2 pathway and increase the production of resolvins and protectin (33). Our study therefore confirms a previous body of evidence showing that MCT-based lipid emulsions *in vitro* affect immune cell functions *in vitro*. Indeed, previous data showed that MCTs, but not LCTs, alter neutrophils functions (17, 34), with inhibitory effects on chemotaxis, phagocytosis, and bacterial killing *in vitro*, as well as in cell signaling (35, 36).

Limitations of the study

Given the severity of our septic shock model, we have deliberately chosen to study only one time point; moreover, repeated blood sampling was impossible because of low blood volume. As the timing to introduce artificial nutrition in critical patients is a matter of debate, and there is still no consensus (37), we assume that the timing of lipid infusion during our experiments could be considered as a limitation of the study.

CONCLUSIONS

This experimental work shows that supplementation by lipid emulsion containing MCTs and LCTs, but neither lipid emulsion containing only LCTs nor 5% dextrose, exacerbates the peritonitis-induced septic shock, via proinflammatory pathway with an intense MP generation reflecting the vascular cell activation. These data could pave the way for further investigations during septic shock in humans.

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