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ORIGINAL ARTICLE

Lipid Emulsions Differentially Affect LPS-Induced Acute Monocytes Inflammation: In Vitro Effects on Membrane Remodeling and Cell Viability

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Abstract The aim of this study was to assess how lipid emulsions for parenteral nutrition affect lipopolysaccharide (LPS)-induced acute monocyte inflammation in vitro. An 18 h long LPS induced human monocyte leukemia cell stimulation was performed and the cell-growth medium was supplemented with three different industrial lipid emulsions: Intralipid®, containing long-chain triglycerides (LCT—soybean oil); Medialipid®, containing LCT (soybean oil) and medium-chain triglycerides (MCT—coconut oil); and SMOFlipid®, containing LCT, MCT, omega-9 and -3 (soybean, coconut, olive and fish oils). Cell viability and apoptosis were assessed by Trypan blue exclusion and flow cytometry respectively. Monocyte composition and membrane remodeling were studied using gas chromatography and NR12S staining. Microparticles released in supernatant were measured by prothrombinase assay. After LPS challenge, both cellular necrosis and apoptosis were increased (threefold and twofold respectively) and microparticle release was enhanced (sevenfold) after supplementation with Medialipid® compared to Intralipid®, SMOFlipid® and monocytes in the standard medium. The monocytes differentially incorporated fatty acids after lipid emulsion challenge. Finally, lipid-treated cells displayed microparticles characterized by disrupted membrane lipid order, reflecting lipid remodeling of the parental cell plasma membrane. Our data suggest that lipid emulsions differentially alter cell viability, monocyte composition and thereby microparticle release. While MCT have deleterious effects, we have shown that parenteral nutrition emulsion containing LCT or LCT and MCT associated to n-3 and n-9 fatty acids have no effect on endotoxin-induced cell death and inflammation.

Keywords Lipid emulsion · Monocyte · Membrane remodeling · Inflammation · Microparticles · Sepsis

Abbreviations

Ctrl	Control
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
IL	Intralipid
LCT	Long-chain triglyceride(s)
LPS	Lipopolysaccharide(s)
MCT	Medium-chain triglyceride(s)
ML	Medialipid
MP	Microparticle
NF-κB	Nuclear factor kappa B

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PtdSer	Phosphatidylserine
PUFA	Polyunsaturated fatty acid(s)
SD	Standard deviation
S	SMOFlipid
THP-1	Human monocyte leukemia cells
TLR4	Toll-like receptor 4

Introduction

Fatty acids, which are a major cell membrane component, are involved in immune and inflammatory processes [1]. Long-chain triglyceride (LCT) n-6 polyunsaturated fatty acids (PUFA) are metabolized into pro-inflammatory arachidonic acid-derived eicosanoids, while eicosanoids derived from n-3 PUFA, mainly eicosapentaenoic acid (EPA), are less pro-inflammatory. In addition, n-3 PUFA enhance anti-inflammatory cytokine release, decrease leucocyte reactive oxygen specie production and lead to resolvin release, contributing to the endogenous inflammation control [2].

Optimizing the composition of lipid emulsions for parenteral nutrition [1–3] is a major challenge and could help to modulate the inflammatory response in critically ill patients, through changes in the cell membrane composition, in eicosanoid and cytokine production and in gene expression [1, 4]. Modulating cell membrane phospholipid composition may indeed alter membrane fluidity and lipid raft organization, and thereby interfere with the activity of key membrane proteins involved in immune cell signaling [5]. Several experimental studies have shown the regulatory role of lipid rafts through the incorporation of functional n-3 PUFA, which limits T cell activation and the immune response [6–8]. Moreover, a concentration-dependent supplementation in DHA has been shown to induce apoptosis and to decrease the proliferation of various cancer cells. Lipid peroxidation and reactive oxygen specie formation would account for these cytotoxic effects [9]. Interestingly, Sutherland et al. [10] have recently demonstrated that the ingestion of n-6 PUFA may acutely alter the endothelial function, resulting in an increased generation of circulating endothelial microparticles. Microparticles are submicron vesicles, released into the extracellular environment, in response to stress, after plasma membrane remodeling and fatty acid randomization [11]. Circulating microparticles behave as vascular and cellular effectors [12–14] and could play a deleterious role by initiating inflammatory processes, apoptosis and multiple organ failure during sepsis in both animals and humans [15–18]. As leucocytes, and more specifically monocytes, are key players in host inflammatory response against the pathogen, the purpose of this study was to assess the role of three different parenteral nutrition lipid emulsions in the modulation of monocyte response and microparticle release in a cell culture model of LPS-stimulated monocytes.

Materials and Methods

Material and Reagents

Cell culture reagents (sodium pyruvate, non-essential amino acids, gentamycin and β-mercaptoethanol, Trypan Blue) and medium (RPMI 1640) were from Biowhittaker (Walkerville, MD, USA). Heated fetal calf serum (complement- and endotoxin-free) was from GIBCO-Life technologies (Cergy-Pontoise, France). Lipopolysaccharide (LPS, *E. coli* serotype O127:B8) was from Sigma-Aldrich (L'isle d'Abeau, Chesne, France). The following industrial lipid-in-water emulsions designed for intravenous infusion were used (see Table I in the *Supplementary Material* for the detailed composition): Intralipid® (soybean oil 200 g/L—LCT) and SMOFlipid® (soybean oil 60 g/L, coconut oil 60 g/L, olive oil 50 g/L, fish oil 30 g/L—LCT, MCT, n-9, n-3) were from Fresenius-Kabi (Bad Homburg, Germany) while Medialipid® (soybean 100 g/L, coconut oil 100 g/L—LCT, MCT) was from B Braun (Melsungen, Germany). Lipid emulsions were prepared in small aliquots and stored under an inert gas atmosphere to avoid oxidation. Antibodies and control immunoglobulins were from Beckman-Coulter (Brea, CA, USA), Jackson (Suffolk, UK) and Invivogen (Toulouse, France).

Cell Culture and Lipid Emulsions Challenge

THP-1 (ATCC TIB-202) human monocyte leukemia cells, obtained from the American Type Culture Collection (Rockville, MR, USA) were cultured in a RPMI 1640 medium supplemented with 10 % (v/v) fetal calf serum, 1 mM sodium pyruvate, 100 mM non-essential amino acids, 10 μg/mL gentamycin and 20 μM β-mercaptoethanol (0.02 % v/v). THP-1 were challenged or not with LPS (15 μg/mL) for 18 h, in medium containing the different industrial lipid emulsions.

Preliminary cell culture experimentations were set to determine the concentrations of triglycerides with minimum toxicity. Cell growth was assessed in culture medium supplemented with each lipid emulsion using final triglycerides concentrations ranging from 0 to 20 g/L and compared to standard medium. A triglyceride (in emulsion) concentration of 0.5 g/L (0.05 % v/v) was selected, because it enabled an optimal cellular growth and a response to LPS. The composition of culture medium (without cells) was analyzed in the presence or absence of

0.5 g/L of triglycerides, leading to a 20-fold increase in triglyceride concentration, whereas cholesterol, protein and glucose concentrations remained unaffected (Table I in the *Supplementary Material)*. In our model, the volume of lipid emulsion added to the medium was limited, there was no significant difference in pH and osmolality in medium supplemented with the different lipid emulsions. In addition, although their fatty acid composition differs, the total molecular weight of the different emulsions was not different either (data not shown).

Measurement of Microparticles

Microparticles were isolated from cell supernatants after a double centrifugation step at 600×*g* for 7 min to remove cells and debris, then at 13,000×*g* for 45 min. A ring of lipids was present at the top of supernatants immediately removed by gentle aspiration. Then, the supernatant was discarded and the microparticle pellet was further concentrated in 0.9 % NaCl or sterile Hank's balanced salt solution supplemented with 1 mM $CaCl_2$. Microparticles were captured on insolubilized Annexin-V and their PtdSer content was measured by prothrombinase assay as previously described [19]. Blood clotting factors (11.2 pM FXa, 33.3 pM FVa and 1.2 μM FII—Hyphen Biomed, Neuville sur Oise France) and calcium (2.2 mM $CaCl_2$) concentrations were set to ensure that PtdSer is the rate-limiting parameter in the generation of soluble thrombin from prothrombin [19]. Results were standardized and expressed as nanomolar PtdSer equivalents (nM Eq. PtdSer) for 10^5 cells by reference to a standard curve constructed with liposomes of known composition and concentration [20].

Cell Necrosis and Apoptosis

Cell necrosis was assessed through Trypan Blue staining (0.2 % v/v final) and cell count. Apoptosis was evaluated by flow cytometry using propidium iodide (Sigma-Aldrich, L'isle d'Abeau, Chesne, France) (100 μg/mL) labeling in ethanol-permeabilized cells [21]. Samples were analyzed using a Beckton Dickinson FACScan flow cytometer (San Jose, CA, USA). Data acquisition (10,000 events per sample) and analysis were carried out using Cell Quest™ software (Becton–Dickinson). The forward and side light scatter and fluorescence channels were set as logarithmic gains.

Gas Chromatography

Total fatty acids were extracted from each cell pellet resuspended in PBS buffer, using a chloroform/methanol mixture (1:1, v/v). C17:0 was added as an internal standard. The extract was washed with a Hank's balanced salt solution to remove proteins. The chloroform phase was concentrated under a stream of nitrogen and redissolved in chloroform/methanol. The dry residue was dissolved with HCl methanolic to generate fatty acid methyl esters (FAME). FAME were then separated from non methylated fatty acids by thin-layer chromatography on silica gel plates (Merck 5721) using dichloromethane as the developing solvent [22]. The plates were sprayed with bromophenol blue and individual bands of methyl ester lipids were scraped off into separate tubes. Methyl esters were removed by 900 μl hexane and analyzed by gas–liquid chromatography on a Trace GC-ThemoFisher gas chromatograph (Thermo Separation Products, Les Ulis, France) equipped with a CP-SIL fused silica capillary column (25 m × 0.25 mm internal diameter) coated with 100 % cyanopropyl siloxane 88 phase 0.2 μm (Chrompack, Les Ulis, France) with helium as the carrier gas and a split ratio of 1:20.

The initial temperature was 120 °C for 1 min, then brought to 20 °C/min up to 165 °C, then up to 210 and 250 °C thereafter. Identification of individual methyl ester components was made by frequent comparisons of the retention times with those of suitable standards. The results were expressed in mol%.

Staining of Plasma and Microparticle Membranes with NR12S and Fluorescence Spectroscopy

Nile Red 12S (NR12S), a probe that assesses the lipid order of model and cellular membranes, was synthesized as previously reported [23, 24]. To stain the cell or microparticle suspensions with the NR12S probe, an appropriate aliquot of NR12S stock solution in DMSO (Sigma-Aldrich, L'isle d'Abeau, Chesne, France) was added to 0.5 mL Hank's balanced salt solution. After vortexing, the solution was immediately added to 0.5 mL of the cell or microparticle suspension to obtain a final probe concentration of 50 nM (<0.25 % DMSO). The final cell concentration after three washes with Hank's balanced salt solution was 1×10^6 cells/mL. Microparticles (20 nM Eq. PtdSer) were also stained after three washings (13000×*g*, 1 h) and a filtering using a Millipore® filter (0.1 μm). All staining procedures were performed using a probe prepared less than 1 min earlier in Hank's balanced salt solution to circumvent the slow aggregation of the probe in water. Before measurements, the cell or microparticle suspension was incubated with the probe for 7 min at room temperature in the dark.

Absorption spectra were recorded on a Cary 4 spectrophotometer (Varian) and fluorescence spectra on a FluoroMax 3.0 (Jobin–Yvon, Horiba) spectrofluorometer. Fluorescence emission spectra were systematically recorded at a 530 nm excitation wavelength at room temperature. All the spectra were corrected from the fluorescence

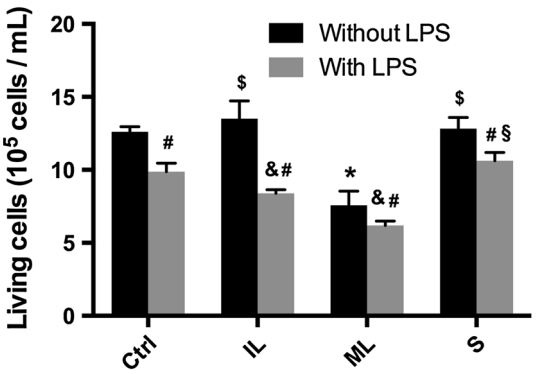

Fig. 1 Monocyte viability in non-stimulated and LPS-stimulated conditions, in standard medium (Ctrl), or in medium supplemented with lipid emulsions containing long-chain triglycerides (IL—Intralipid®), or a mixture of medium and long-chain triglycerides only (ML—Medialipid®) or associated with omega 9 and 3 (S—SMOFlipid®). Trypan blue staining, $n = 4$, ±SD. $^{*}p < 0.05$ *vs* Ctrl, $^{\&}p < 0.05$ *vs* Ctrl-LPS, $^{\#}p < 0.05$ *vs* condition without LPS, $^{\$}p < 0.05$ *vs* ML, $^{\S}p < 0.05$ *vs* ML-LPS

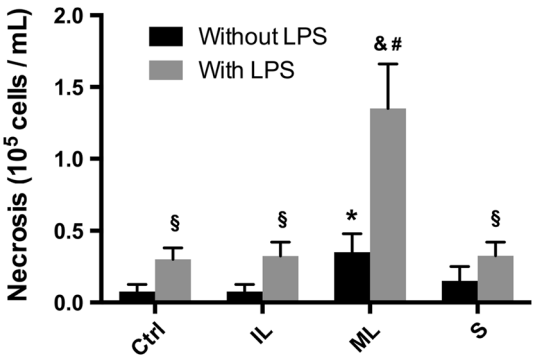

Fig. 2 THP-1 necrosis in non-stimulated and LPS-stimulated conditions, in standard medium (Ctrl), or in medium supplemented with lipid emulsions containing long chain triglycerides (IL—Intralipid®), or a mixture of medium and long chain triglycerides only (ML—Medialipid®) or associated with omega 9 and 3 (S—SMOFlipid®). Trypan blue staining, $n = 4$, ±SD. $^{*}p < 0.05$ *vs* Ctrl, $^{\&}p < 0.05$ *vs* Ctrl-LPS, $^{\#}p < 0.05$ *vs* condition without LPS, $^{\S}p < 0.05$ *vs* ML-LPS

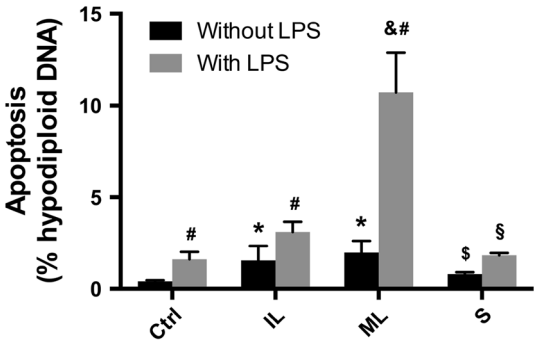

Fig. 3 THP-1 cell apoptosis in non-stimulated and LPS-stimulated conditions, in standard medium (Ctrl), or in medium supplemented with lipid emulsions containing long-chain triglycerides (IL—Intralipid®), or a mixture of medium and long-chain triglycerides only (ML—Medialipid®) or associated with omega 9 and 3 (S—SMOFlipid®). Flow cytometry, $n = 8$, ±SD. $^{*}p < 0.05$ *vs* Ctrl, $^{\&}p < 0.05$ *vs* Ctrl-LPS, $^{\#}p < 0.05$ *vs* condition without LPS, $^{\$}p < 0.05$ *vs* ML, $^{\S}p < 0.05$ *vs* ML-LPS

of the corresponding blank (suspension of cells or microparticles without the probe).

Statistic

In vitro experiments were performed three to twelve times. The non-parametric Kruskal–Wallis test was used for comparison of in vitro measurements between the groups. Whenever a significant difference was found between groups, subsequent Dunn post hoc tests were performed. All values are presented as means ± SD for *n* experiments; with *n* representing the number of experiments. All statistics were performed with the Statview™ software (version 5.0; SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant.

Results

Medium-Chain Triglycerides Decrease Cell Viability and Increase LPS-Induced Cell Mortality

Lipid emulsions containing MCT or a mixture of LCT and MCT significantly impaired the viability of LPS-treated cells (control-LPS) in a time- and concentration-dependent manner (Figs. 1a, b, 2a, b in the *Supplementary Material*), mainly after supplementation with MCT (Medialipid®) (Intralipid®: $8.4 \pm 0.2 \times 10^5$ cells/mL or Medialipid®: $6.2 \pm 0.3 \times 10^5$ cells/mL *vs* control-LPS: $9.9 \pm 0.6 \times 10^5$ cells/mL, $p < 0.05$) (Fig. 1). The emulsion containing a mixture of MCT/LCT/n-9/n-3 (SMOFlipid®) had no effect on cell viability, which was comparable to values obtained in LPS-treated cells (SMOFlipid®: $10.6 \pm 0.6 \times 10^5$ cells/mL *vs* control-LPS: $9.9 \pm 0.6 \times 10^5$ cells/mL) (Fig. 1).

In non-stimulated conditions (control without LPS), MCT supplementation (Medialipid®) significantly increased cell necrosis ($0.4 \pm 0.1 \times 10^5$ cell/mL *vs* control without LPS $0.1 \pm 0.1 \times 10^5$ cells/mL) and apoptosis (2.0 ± 0.6 % *vs* control without LPS 0.4 ± 0.1 %) ($p < 0.05$). A similar deleterious effect of MCT was observed after LPS stimulation, with a high degree of induced necrosis ($1.4 \pm 0.3 \times 10^5$ cells/mL *vs* control-LPS $0.3 \pm 0.1 \times 10^5$ cells/mL) and apoptosis (10.7 ± 2.2 % *vs* control-LPS 1.6 ± 0.4 %) ($p < 0.05$). In contrast, LCT supplementation (Intralipid®) had no effect on LPS-induced cell mortality (Figs. 2, 3). Similarly, the emulsion containing a mixture of MCT/LCT/n-9/n-3

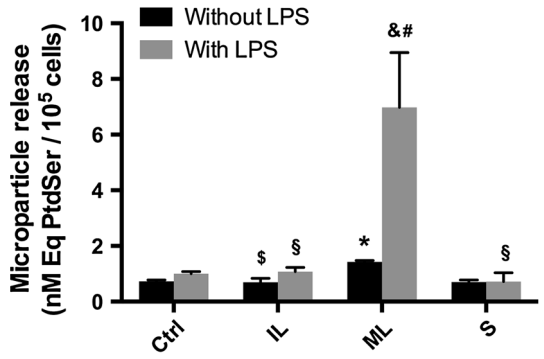

Fig. 4 Release of procoagulant microparticles non-stimulated and LPS-stimulated conditions in standard medium (Ctrl), or in medium supplemented with lipid emulsions containing long-chain triglycerides (IL—Intralipid®), or a mixture of medium and long-chain triglycerides only (ML—Medialipid®) or associated with omega 9 and 3 (S—SMOFlipid®). Prothrombinase assay, $n = 4$, ±SD. $^{*}p < 0.05$ *vs* Ctrl, $^{\&}p < 0.05$ *vs* Ctrl-LPS, $^{\#}p < 0.05$ *vs* condition without LPS, $^{\$}p < 0.05$ *vs* ML, $^{\S}p < 0.05$ *vs* ML-LPS

(SMOFlipid®) had no significant effect on cell necrosis and apoptosis, compared to the control, whether cells were treated with LPS or not (Figs. 2, 3).

Medium-Chain Triglycerides Increase LPS-Induced Procoagulant Microparticle Release

Medium-chain triglycerides (Medialipid®) significantly increased the generation of procoagulant microparticle in the absence of LPS challenge (1.4 ± 0.1 nM Eq PtdSer/10^5 cells *vs* control 0.7 ± 0.1 nM Eq PtdSer/10^5 cells, $p < 0.05$), as well as in the presence of LPS (7.0 ± 2.0 nM Eq PtdSer/10^5 cells *vs* control-LPS 1.0 ± 0.1 nM Eq PtdSer/10^5 cells, $p < 0.05$). Microparticle release remained unaffected when cells were challenged with LCT (Intralipid®) or with the mixed emulsion containing MCT/LCT/n-9/n-3 (SMOFlipid®), whether associated with LPS or not (Fig. 4).

LPS Affects Monocyte Fatty Acid Composition

We investigated monocyte fatty acid composition, using gas chromatography. We showed that LPS stimulation significantly decreased the integration of most fatty acids into the cell (C16.0, C18.0, C18.1 n-9, C18.1 n-7, C18.2, C18.3, C20.4 n-6 and C22.6 n-3) compared to non-stimulated monocytes (Table 1).

Monocytes Incorporate Medium-Chain Fatty Acids after Lipid Emulsion Challenge

C8.0 and C10.0 were neither detected in monocytes in basal or endotoxinic conditions (THP-1 or THP-1 + LPS), nor in monocytes treated with MCT-free lipid emulsions. Cell exposure to lipid emulsions containing MCT (Medialipid® and SMOFlipid®), whether associated with LPS stimulation or not, led to C8.0 and C10.0 incorporation, independently of the MCT content in the lipid emulsions (Table 1).

Parenteral Lipid Emulsions Alter Monocyte Composition in Long-Chain Fatty Acids

Treating monocytes with lipid emulsions affected their content in bioactive fatty acids, with a significant increase in C16.0, C.18.0, C18.1 n-9 and C18.2 in non-stimulated and LPS-stimulated conditions, compared to untreated monocytes (Table 1).

Compared to other lipid emulsions, the emulsion containing only LCT (Intralipid®) prompted an eight to ninefold increase in C18.2 and a two to threefold rise in C18.3 in non-stimulated and LPS-stimulated states, respectively. Compared to LPS-stimulated monocytes, the emulsion containing a mixture of MCT/LCT/n-9/n-3 (SMOFlipid®) significantly increased the amount of C18.2, but this increase was significantly lower than with Intralipid® and Medialipid® (Table 1).

Treatment by Lipid Emulsions Decreases the Plasma Membrane Lipid Order of Parental Cells and Has Major Consequences on the Membrane Structure of Derived Microparticles

In intact cell suspensions, the maximum emission observed after staining with NR12S was centered at 595 nm. The microparticles harvested from the supernatant of such cells (THP and THP-LPS) showed a slightly red shifted spectra, indicating that the formation of microparticles was associated with a small decrease in the membrane order. Remarkably, the treatment of cells with any mixture of unsaturated lipids modified the spectrum of released microparticles that was characterized by a significant red shifted emission of NR12S centered at 604–605 nm, close to that observed in model membranes of pure disordered phase (Fig. 5).

Discussion

Fatty acid supplementation leads to plasma membrane remodeling and may thus affect immune functions with clinical consequences in patients with inflammatory diseases. In the present study, we developed a cell culture model using LPS-stimulated monocytes and assessed the effects of a lipid emulsion-enriched medium on acute inflammatory stress and cell membrane remodeling.

Table 1 Monocyte fatty acid composition after treatment with lipid emulsions containing long-chain triglycerides (IL—Intralipid®), a mixture of medium- and long-chain triglycerides only (ML—Medialipid®) or associated with omega 9 and 3 (S—SMOFlipid®)

Fatty acids	Ctrl	IL	ML	S	Ctrl + LPS	IL + LPS	ML + LPS	S + LPS
C8.0	0 ± 0	0 ± 0	9.3 ± 1.0**	12.7 ± 1.9**	0 ± 0	0 ± 0	9.8 ± 1.4**#	11.9 ± 0.3**#
C10.0	0 ± 0	0 ± 0	2.2 ± 0.6**	2.9 ± 0.5**	0 ± 0	0 ± 0	2.6 ± 0.6**#	6.0 ± 2.5**#
C12.0	2.3 ± 0.3	1.4 ± 0.0	2.4 ± 0.5	1.4 ± 0.2	2.5 ± 1.1	1.7 ± 0.6	2.0 ± 0.5	2.0 ± 0.7
C14.0	3.1 ± 1.0	1.4 ± 0.2	1.9 ± 0.4	2.0 ± 0.4	3.4 ± 0.3	1.5 ± 0.2	1.8 ± 0.4	1.9 ± 0.2
C16.0	36.1 ± 2.1	21.1 ± 1.2**	22.6 ± 1.1**	21.3 ± 1.1**	29.7 ± 2.2**	22.6 ± 1.0**#	22.7 ± 1.6**	20.5 ± 0.5**
C16.1	4.6 ± 1.8	2.1 ± 0.2	2.6 ± 0.6	3.1 ± 0.2	4.4 ± 0.6	2.0 ± 0.2	2.4 ± 0.2	3.3 ± 0.4
C18.0	11.3 ± 2.3	10.6 ± 0.6**	11.1 ± 0.5**	9.7 ± 0.5**	13.6 ± 1.5	9.9 ± 0.6**	9.6 ± 1.2	8.6 ± 1.3
C18.1 n-9	15.6 ± 2.5	16.3 ± 1.5**	17.3 ± 0.8**	19.8 ± 1.1**	17.2 ± 0.7	17.9 ± 1.4**#	16.8 ± 2.5**#	20.0 ± 2.5**#
C18.1 n-7	7.3 ± 1.7	7.3 ± 0.5**	5.3 ± 0.7	5.4 ± 0.2	9.2 ± 1.5	3.1 ± 0.5#	3.4 ± 0.4#	3.5 ± 0.6#
C18.2 n-6	4.4 ± 2.1	27.5 ± 0.8**	14.3 ± 0.7**	10.0 ± 0.8**	3.5 ± 0.3**	29.4 ± 1.1**#	18.6 ± 1.6**#	11.4 ± 0.7**#
C18.3 n-3	0.8 ± 0.6	2.8 ± 0.3**	1.0 ± 0.0	0.8 ± 0.2	1.3 ± 0.3	3.6 ± 0.8**#	1.5 ± 0.2	1.1 ± 0.2
C20.4 n-6	6.5 ± 0.8	4.6 ± 0.4	5.0 ± 0.5	5.0 ± 0.3	7.2 ± 0.9	4.1 ± 0.3	4.5 ± 0.5	4.8 ± 0.5
C20.5 n-3	2.3 ± 1.9	1.4 ± 0.2	1.3 ± 0.3	1.8 ± 0.2	2.3 ± 0.2	1.1 ± 0.1	1.2 ± 0.7	1.6 ± 0.5
C22.6 n-3	5.5 ± 0.9	3.6 ± 0.3	3.6 ± 0.6	4.1 ± 0.3	5.6 ± 0.6	3.0 ± 0.1	3.2 ± 0.6	3.6 ± 0.8

Gas chromatography, ** $p < 0.01$ *vs* Ctrl, # $p < 0.01$ *vs* Ctrl + LPS, $n = 6$, mol% ±SD

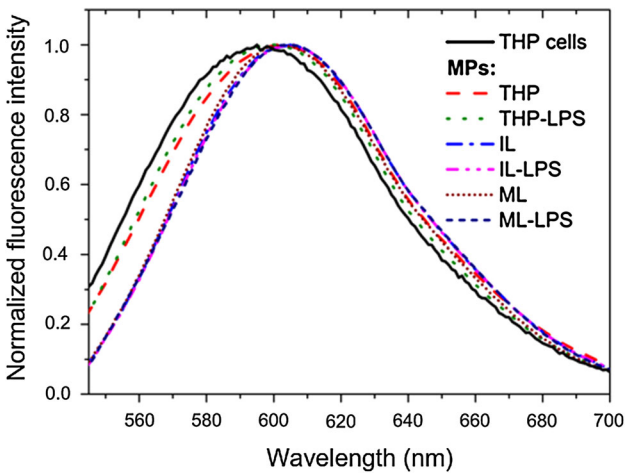

Fig. 5 Normalized fluorescence spectra of NR12S (50 nM) in intact cells (THP cells) and in microparticles (MP) after treating cells with lipid emulsions containing long-chain triglycerides (IL—Intralipid®), or a mixture of medium and long-chain triglycerides (ML—Medialipid®), in the presence or absence of LPS. $n = 3$

First, our data suggest that MCT supplementation specifically blunt monocyte survival in a time- and concentration-dependent manner. These results are consistent with previous data showing that MCT, but not LCT, alter neutrophil functions [25, 26], with inhibitory effects on chemotaxis, phagocytosis, and bacterial killing in vitro [27, 28]. In our model only small volumes of lipid emulsions were added to the medium (0.05 %), thereby excluding any unspecific pH and osmolality effects. Wanten et al. [29] showed that MCT induced oxygen radical production in unstimulated neutrophils, contrary to LCT. Oxidative stress could therefore trigger increased cell injury and mortality. The lipid emulsions contain different amounts of α-tocopherol (vitamin E), which are used to prevent lipid peroxidation and could act as a pharmacological modulator for inflammatory diseases. Interestingly, the vitamin E concentration is nearly six times higher in Medialipid® and SMOFlipid® than in Intralipid® (Table I in *Supplementary Material*). In our present experiments, vitamin E antioxidative properties are not likely to account for the deleterious effects of Medialipid®, compared to Intralipid® or SMOFlipid®.

LPS challenge led to both cellular necrosis and apoptosis in medium enriched with emulsions containing MCT (Medialipid®), while a mixture of MCT/LCT/n-9/n-3 (SMOFlipid®) did not promote significant necrosis and seemed to be more neutral towards cells. These data are consistent with previous studies, showing that n-3 PUFA have beneficial effects, notably through the modulation of the immune response [30]. We have thus established MCT as pro-apoptotic agents. The enhanced apoptosis evidenced in the present work is also in line with previous studies demonstrating that supplementing the culture medium with fatty acids induces cell apoptosis [31, 32].

As expected from numerous data, microparticle generation by THP-1 was correlated with apoptosis [33]. Microparticles were shown to induce macrophage apoptosis by enhancing the free non-esterified arachidonic acid pool, resulting in an alteration of membrane lipid homeostasis [34]. Interestingly, we have shown that the release of microparticle is not increased by n-3 fatty acids, in accordance with previous data showing that platelet- and monocyte-derived microparticle levels are significantly

lower in post-myocardial infarction patients treated by n-3 fatty acids [35]. Since MCT lipid emulsions led to increased procoagulant microparticle release, one may hypothesize that MCT more dramatically interfere with cell membrane composition and might deeply modify cell membrane composition and structure.

It is well known that in a culture medium deprived of essential fatty acids, glucose induces lipogenesis and high rates of *de novo* fatty acid biosynthesis [36], which may further take part in the cellular metabolism. We have shown how supplementing the medium with lipid emulsions induces changes in monocyte composition, with differential incorporation of MCT and LCT. Although MCT are hydrosoluble and mainly have an energetic function, our data have shown that a proportion of MCT is incorporated into the monocyte and involved in apoptosis and microparticle generation. Finally, some fatty acids like C18.3, which is increased in the monocyte after LCT treatment, might have some beneficial effects with subsequent decreased necrosis and apoptosis. It has indeed been previously shown that α-linolenic acid (C18.3, n-3) has anti-oxidative and anti-inflammatory properties, leading to decreased apoptosis in different cell types [37–40].

The present study also demonstrates that LPS interferes with the action of fatty acids from parenteral nutrition. First, we have shown that without any lipid supplementation, some fatty acid chains are decreased in cell after LPS challenge, which may be due to fatty acid consumption for eicosanoid synthesis and inflammatory processes initiation. Secondly, fatty acids are differentially incorporated into the cell after LPS stimulation. The inflammatory response induced by LPS is a major mechanism of host-pathogen interactions, responsible for the secretion of pro-inflammatory cytokines and induction of apoptosis [41]. Fatty acids alter TLR4 signaling pathways, through lipid raft modulation. Kim et al. [42] have indeed recently shown that TLR4 receptor recruitment into lipid rafts is modulated by n-3 fatty acids, leading to the down-regulation of TLR4-mediated signaling. The anti-inflammatory actions of n-3 fatty acids would include the modulation of cellular membranes and lipid raft composition, the inhibition of nuclear factor kappa B (NF-κB) transcription with a subsequent decrease in inflammatory genes expression and the activation of anti-inflammatory transcription factors [43, 44]. It has also recently been shown that fish oil alters innate and adaptive B cell function, by increasing the membrane order and the size of lipid rafts, both effects being responsible for functional changes [45, 46]. In our study, we have shown that the plasma membrane order is altered by a lipid emulsion treatment. Indeed, the maximum emission of the spectrum observed in intact cells is centered at 595 nm, an intermediate value observed between the ordered (572 nm) and disordered (605 nm) phases of model lipid membranes [23], which was expected given that the outer membrane leaflet consists of lipids forming ordered and disordered phases [47, 48]. Interestingly, THP-1 cell treatment by lipid emulsions produced a limited decrease in the lipid order of the cell plasma membrane, in accordance with the limited apoptosis and microparticle generation in non-stimulated conditions. Strikingly, microparticles released from lipid-treated cells showed subsequent high disorder in their membranes. It is thus tempting to hypothesize that after the action of exogenous fatty acids, the cells generate microparticles from those parts of the plasma membranes that present a liquid disordered phase. In this way, the cells might preserve a relatively high order of their plasma membrane even after treatment with unsaturated lipids.

Conclusions

In this cell culture model of LPS-stimulated monocytes, we have shown that MCT decrease monocyte growth, increase their mortality and stimulate the subsequent procoagulant microparticle release. These effects may be partly explained by MCT-induced cell membrane disorders. In contrast, emulsions containing LCT or MCT/LCT/n-9/n-3 seemed to be more neutral, without deleterious effects on monocyte viability, membrane remodeling and MP shedding.

Study Limitations

How fatty acids have an effect on membrane fluidity and metabolic pathways remains to be elucidated by in vitro studies. The clinical relevance of the results of this study using parenteral nutrition in septic shock has yet to be established by in vivo studies.

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Conflict of interest The authors declare no conflict of interest.

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