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*J PEN J Parenter Enteral Nutr* 2012 36: 677 originally published online 26 January 2012
DOI: 10.1177/0148607111432759

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What is This?
Human Leukocyte Death After a Preoperative Infusion of Medium / Long-Chain Triglyceride and Fish Oil Parenteral Emulsions: A Randomized Study in Gastrointestinal Cancer Patients

Maria Fernanda Cury-Boaventura, PhD1; Raquel Susana Matos de Miranda Torrinhas, MB2; André Borella Pereira de Godoy3; Rui Curi, PhD3; and Dan Linetzky Waitzberg, PhD2

Abstract

Background: Parenteral lipid emulsions (LEs) can influence leukocyte functions. The authors investigated the effect of 2 LEs on leukocyte death in surgical patients with gastrointestinal cancer. Material and Methods: Twenty-five patients from a randomized, double-blind clinical trial (ID: NCT01218841) were randomly included to evaluate leukocyte death after 3 days of preoperative infusion (0.2 g fat/kg/d) of an LE composed equally of medium/long-chain triglycerides and soybean oil (MCTs/LCTs) or pure fish oil (FO). Blood samples were collected before (t0) and after LE infusion (t1) and on the third postoperative day (t2). Results: After LE infusion (t1 vs t0), MCTs/LCTs did not influence cell death; FO slightly increased the proportion of necrotic lymphocytes (5%). At the postoperative period (t2 vs t0), MCTs/LCTs tripled the proportion of apoptotic lymphocytes; FO maintained the slightly increased proportion of necrotic lymphocytes (7%) and reduced the percentage of apoptotic lymphocytes by 74%. In the postoperative period, MCT/LCT emulsion increased the proportion of apoptotic neutrophils, and FO emulsion did not change any parameter of apoptosis in the neutrophil population. There were no differences in lymphocyte or neutrophil death when MCT/LCT and FO treatments were compared during either preoperative or postoperative periods. MCT/LCTs altered the expression of 12 of 108 genes related to cell death, with both pro- and antiapoptotic effects; FO modulated the expression of 7 genes, demonstrating an antiapoptotic effect. Conclusion: In patients with gastrointestinal cancer, preoperative MCT/LCT infusion was associated with postoperative lymphocyte and neutrophil apoptosis. FO has a protective effect on postoperative lymphocyte apoptosis. (JPEN J Parenter Enteral Nutr. 2012;36:677-684)

Keywords

lips; fatty acids; parenteral nutrition; trauma

Clinical Relevancy Statement

Commercial parenteral lipid emulsions (LEs) containing medium/long-chain triglycerides (MCTs/LCTs) or based in fish oil (FO) were designed to provide less amounts of n-6 polyunsaturated fatty acids (PUFAs) and higher n-3 PUFAs, respectively, than that provided by standard soybean oil LE (SO), as alternatives to minimize the risks associated with it, including potentially inflammatory and immunosuppressive effects.

Leukocyte death through apoptosis plays an important role for immune dysfunction observed in trauma and sepsis. We showed that, in surgical patients with gastrointestinal cancer, preoperative MCT/LCT infusion induced or did not protect against postoperative lymphocyte and neutrophil apoptosis, whereas FO has a protective effect on postoperative lymphocyte apoptosis.

Introduction

Lipid emulsions (LEs) are an integral part of parenteral nutrition (PN). As a source of nonglucidic energy and essential fatty acids, they contribute to the prevention of several metabolic disturbances associated with the intravenous (IV) feeding of amino acids and glucose alone.1,2

The first well-tolerated LE was based on soybean oil (SO). SO is rich in long-chain ω-6 polyunsaturated fatty acids (PUFAs), which are precursors of potentially inflammatory eicosanoids. Several authors have described the impairment of neutrophil, lymphocyte, monocyte, and macrophage functions, from the 1Institute of Physical Activity and Sports Science, Cruzeiro do Sul University, São Paulo, Brazil; 2Department of Gastroenterology, Faculty of Medicine, University of São Paulo (LIM 35), São Paulo, Brazil; and 3Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

Financial disclosure: This research was supported by CNPq, FAPESP (08/00163-9), and CAPES.

Received for publication July 23, 2011; accepted for publication August 9, 2011.

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including leukocyte death, in the context of parenteral therapy with SO emulsions.\textsuperscript{2,3-7}

The impairment of leukocyte functions by SO emulsion may be related to the induction of leukocyte death. An excess of α-6 PUFA raises the production of eicosanoids, reactive oxygen species (ROS), and reactive nitrogen species (RNS) and can cause cell death.\textsuperscript{1,8,9} We previously reported that SO emulsion could impair immune function by decreasing lymphocyte proliferation and leading to neutrophil and lymphocyte apoptosis and necrosis in healthy volunteers.\textsuperscript{7}

New LEs with different fatty acid compositions were designed for clinical use, aiming to decrease the amount of long-chain α-6 PUFAs provided by SO. These LEs were achieved by diluting SO with medium-chain triglycerides (MCTs) or by creating LEs rich or based in long-chain ω-3 PUFAs. Commercial LEs containing 50% MCT and 50% SO (MCT/long-chain triglyceride [LCT]) may have a reduced impact on immune response because MCTs do not lead to eicosanoid synthesis and are not susceptible to lipid peroxidation.\textsuperscript{2}

Commercial lipid emulsion composed of fish oil (FO) contains a large amount of long-chain ω-3 PUFAs, which synthesize fewer inflammatory eicosanoids than α-6 PUFA and have been shown to exert several other anti-inflammatory effects.\textsuperscript{2}

Major abdominal surgery can lead to an intense systemic inflammatory response, which, in turn, may increase the risk of postoperative complications and multiple organ failure. Leukocyte death through apoptosis plays an important role for immune dysfunction and multiple organ failure observed in inflammatory conditions, such as trauma and sepsis.\textsuperscript{10,11} Proinflammatory cytokines, such as interleukin (IL)–6, released during the surgical stress response, can induce leukocyte apoptosis and impair immune function.\textsuperscript{12,13}

In surgical patients, MCT/LCT emulsion prevented the impairment of bacterial killing activity of neutrophils and resulted in increased numbers of blood peripheral lymphocytes, in relation to SO emulsion, and FO emulsion preserved the expression of leukocyte antigen HLA-DR and lymphocyte distribution and proliferation, prevented the postoperative drop in interferon (IFN)–γ release compared with SO emulsion, and also increased IL-2, tumor necrosis factor (TNF)–α, and IL-2R compared with PN without fat.\textsuperscript{16,17}

To assess whether these positive findings regarding MCT/LCT and FO emulsions on leukocyte functions and counts in surgical patients are associated with a possible protective effect on leukocyte death, the impact of these LEs on cell viability, apoptotic markers, and expression of genes associated with cell death was investigated here in peripheral leukocytes from surgical patients with gastrointestinal cancer.

Materials and Methods

Ethical Issues

The current study was carried out following the ethical recommendations of the Declaration of Helsinki and the Ethical Committee of the University of São Paulo “CAPPesq—Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP),” which approved the study protocol. All enrolled patients provided informed consent prior to the commencement of the study.

Patient Selection

This prospective study evaluated 25 patients who were randomly selected from surgical patients with stomach or colon cancer enrolled in a larger study by the Department of Surgical Gastroenterology of the HCFMUSP, which was registered in the Clinical Trials Database (ID: NCT01218841). Patient selection aimed to include the first 10 patients allocated in each treatment arm of this large study who had successfully concluded the LE infusion protocol. Patients were adults (30–75 years old), had Karnofsky indices ≥60, and good venous condition suitable for parenteral therapy and blood collection. Exclusion criteria were intolerance or allergy to any ingredient of LE; diagnosis of infectious (ie, acquired immune deficiency syndrome), inflammatory (ie, arthritis), immunologic (ie, lupus), or metabolic diseases (ie, insulin-dependent diabetes); dementia; medicine intake that could significantly modulate metabolism; and implanted electromagnetic instruments.

LE Infusion Protocol

The infusion of MCT/LCT (Lipovenos MCT 10% Fresenius Kabi, Bad Homburg, Germany) or FO (Omegeavenos 10%, Fresenius Kabi) was performed in a double-blind manner. Infusion was performed for 3 days before surgery, at 0.2 g fat/kg body weight per day, for 6 hours continuously and with daily changes of exclusive peripheral venous access. Independent pharmacists (from Farmoterápica, SP Brazil) prepared LE bags according to a computer-generated randomized list.

Cell Isolation

Immediately before the start of LE infusion (basal, t0), at the end of LE infusion (preoperative, t1), and on the third postoperative day (postoperative, t2), peripheral blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA; 1 mg/mL) and analyzed for leukocyte death at the Department of Physiology and Biophysics, Institute of Biomolecular Sciences, University of Sao Paulo. Peripheral blood mononuclear cells (PBMCs; a mixture of monocytes and lymphocytes) and neutrophils were isolated by Ficoll-Hypaque density gradient centrifugation. Blood was diluted in phosphate-buffered saline (PBS; 1:1), added to a BD Falcon conical tube (BD Biosciences, San Diego, CA) containing Histopaque-1077, and centrifuged (Harrier 18/80; Sanyo, London, UK) for 30 minutes at 800 g and 4°C. PBMCs were collected from the interface and washed once with PBS.
Neutrophils were isolated from the inferior sediment after erythrocytes were lysed with lysis buffer (150 mM NH₄Cl, 10 mM NaHCO₃, 0.1 mM EDTA, pH 7.4). The PBMCs remained in RPMI 1640 medium for 30 minutes to allow monocytes to adhere to the plates. Next, the supernatant medium containing lymphocytes was collected to obtain a pure lymphocyte preparation (approximately 98%)..

**Cell Viability**

Cells were centrifuged at 1000 g for 15 minutes at 4°C, and the obtained pellet was resuspended in 500 µL of PBS. Next, 50 µL of propidium iodide (PI) solution (50 mg/mL in PBS) was added, and the cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences). PI is a highly water-soluble fluorescent compound that is excluded from viable cells. In cells that have lost membrane integrity, PI binds to DNA by intercalating between the bases with little or no sequence preference. Cell fluorescence was measured using the FL2 channel (orange-red fluorescence, 585/42 nm). Ten thousand events were analyzed per experiment. Cells with PI fluorescence were then evaluated using Cell Quest software (Becton Dickinson, Franklin Lakes, NJ).

**DNA Fragmentation**

DNA fragmentation was determined by flow cytometry after DNA staining with PI according to the method described by Nicoletti et al. Cells were resuspended in a solution containing detergents to allow the prompt incorporation of the dye into DNA. Briefly, cells were centrifuged at 1000 g for 15 minutes at 4°C. The pellet was gently resuspended in 300 µL hypotonic solution containing 50 µ/mL PI, 0.1% sodium citrate, and 0.1% Triton X-100. The cells were then incubated for 2 hours at room temperature. Fluorescence was determined by flow cytometry, as described above.

**Phosphatidylserine Externalization**

Phosphatidylserine (PS) externalization was analyzed by flow cytometry after PS staining with annexin V–FITC, according to the method described by Vermes et al. Cells (1 × 10⁶) were washed twice with cold PBS and resuspended in 100 µL binding buffer (10 mM HEPES/NaOH, 140 mM NaCl, 2.5 mM CaCl₂); then, 5 µL of fluorescein-conjugated annexin V (annexin V–FITC) was added. Cells were gently agitated and incubated for 15 minutes in the dark at room temperature (20–25°C). Next, 10 µL of PI solution and 400 µL of binding buffer were added and cells were analyzed by flow cytometry. Annexin V–FITC fluorescence was measured in the FL1 channel (green fluorescence, 530/30 nm), and PI was measured in the FL2 channel (orange-red fluorescence, 585/42 nm) of a flow cytometer, as described above.

**Mitochondrial Transmembrane Potential**

Cells were centrifuged at 1000 g, for 15 minutes at 4°C, and the pellet was resuspended in 1000 µL PBS. Rhodamine 123 is a cell-permeable, cationic, fluorescent dye that is readily sequestered by active mitochondria without inducing toxic effects. Rhodamine 123 (5 µM) was added and the cells were then incubated for 15 minutes at 37°C in the dark. Cells were washed twice with cold PBS and incubated for 30 minutes at 30°C in the dark. Fluorescence was determined using the FL1 channel (green fluorescence, 530/30 nm) of a flow cytometer, as described above.

**Gene Expression**

Postoperative gene expression was evaluated in lymphocytes by comparing data from t0 and t1 samples and using the Codelink Expression Bioarray System (16-Assay Bioarray Hybridization and Detection) from Amersham Biosciences (Piscataway, NJ). The 16-Assay Bioarray Hybridization and Detection consists of 16 independent arrays and hybridization chambers in the space of a standard glass slide containing 800 genes related to inflammation, of which 108 were involved in cell death. We first obtained total RNA from lymphocytes and bacterial controls. We used the purified sample total RNA to prepare the first-strand cDNA synthesis (42°C, 2 hours) and second-strand cDNA (16°C, 2 hours). We incubated purified cDNA at 37°C for 14 hours to evaluate in vitro transcription and biotin labeling of cRNA. cRNA was fragmented at 94°C for 20 minutes and incubated at 37°C for 18–24 hours for hybridization. The slide was washed, stained with Cy5-streptavidin, and scanned using the GenePix 4000B (Union City, CA, USA). We analyzed the scanned image with Codelink Expression Analysis Software. Only signals that differed from the control by at least 2-fold in 2 independent experiments were considered significant.

**Statistical Analysis**

Results are presented as means ± SEM. The results were compared regarding treatment and time by 2-way analysis of variance (ANOVA) and repeated measures with the Bonferroni posttest (GraphPad Prism version 4.0 for Windows; GraphPad Software, San Diego, CA). The level of significance was set at \( P < .05 \).

**Results**

**Description of Patients**

Patient randomization was considered adequate because no differences in the mean descriptive characteristics (age, gender, type of tumor, nutrition status, type of open surgery, or comorbidities) were found between the patient populations of the 2 groups (\( \chi^2, P \geq .3169; \) Table 1).
Leukocyte Death

The effect of LE infusion on leukocyte death before surgical trauma was assessed by comparing data from t0 and t1. In lymphocytes, MCT/LCT emulsion did not alter cell viability, PS externalization, DNA fragmentation, or mitochondrial depolarization (Figure 1); FO emulsion decreased the proportion of viable cells by 5% (Figure 1A). In neutrophils, LE infusion did not alter cell viability or any parameter of apoptosis.

The impact of preoperative LE infusion on postoperative leukocyte death was determined by comparing data from t0 and t2. In lymphocytes, MCT/LCT emulsion tripled the proportion of cells with DNA fragmentation (Figure 1B), and FO emulsion reduced the relative proportion of viable cells by 7% (Figure 1A). In neutrophils, LE infusion did not alter cell viability or any parameter of apoptosis.

The impact of preoperative LE infusion on postoperative leukocyte death was determined by comparing data from t0 and t2. In lymphocytes, MCT/LCT emulsion tripled the proportion of cells with DNA fragmentation (Figure 1B), and FO emulsion reduced the relative proportion of viable cells by 7% (Figure 1A), reduced the percentage of cells with PS externalization by 74% (Figure 1C), and had no effect on cell DNA fragmentation or mitochondrial depolarization (Figure 1B,D). In postoperative neutrophils, MCT/LCT emulsion did not alter cell viability, DNA fragmentation, or mitochondrial depolarization but increased the proportion of neutrophils with PS externalization (Figure 2). FO emulsion did not change cell viability or any parameter of apoptosis in the neutrophil population (Figure 2).

At the cellular level, there were no differences in lymphocyte or neutrophil death when MCT/MCT and FO treatments were compared during either preoperative or postoperative periods (P > .05).

Postoperative Gene Expression in Lymphocytes

Of 108 genes related to cell death, MCT/LCT altered the expression of 12 genes and FO emulsion modulated the expression of 7 genes (Table 2). MCT/LCT infusion upregulated the expression of 7 genes related to cell death (TNF receptor–associated factor 5 [TRAF5], TNF receptor–associated factor 1 [TRAF1], Fas ligand [TNF superfamily, member 6—FASLG], v-myc myelocytomatosis viral oncogene homolog [avian—MYC], BCL2-like 1 nuclear gene encoding mitochondrial protein [BCL2L1], BCL2-antagonist of cell death [BAD], and melanoma inhibitory activity [MIA]) and 2 genes related to cell proliferation (vascular endothelial growth factor [VEGF] and connective tissue growth factor [CTGF]) and downregulated the expression of 3 genes related to cell death (BH3-interacting domain death agonist [BID], CASP8 and FADD-like apoptosis regulator [CFLAR], and cluster of differentiation 14 [CD 14]).

FO emulsion modulated the expression of 7 genes. FO emulsion upregulated the expression of 3 genes related to cell death (TNF receptor–associated factor 3 [TRAF3], BCL2-associated athanogene 4 [BAG4], and nonmetastatic cells 1 [NME1] protein [NM23A]) and 2 genes related to cell proliferation (macrophage colony-stimulating factor 1 [CSF1] and granulocyte-macrophage colony-stimulating factor 2 [CSF2]) and downregulated the expression of 2 genes related to cell death (bifunctional apoptosis regulator [BFAR] and growth arrest and DNA damage-inducible alpha [GADD45A]).

Discussion

To achieve the effect of MCT/LCT and FO emulsions on leukocyte death under surgical trauma, we evaluated cell viability, apoptotic markers, and expression of genes associated with cell death in peripheral leukocytes from surgical patients with colon and gastric cancer who received a preoperative infusion of these LEs. Our choice to study surgical patients with both
Figure 1. The effect of fish oil (FO) or medium/long-chain triglycerides (MCTs/LCTs) on cell viability (A), DNA fragmentation (B), phosphatidylserine externalization (C), and mitochondrial polarization (D) in lymphocytes. Lymphocytes were freshly isolated from the peripheral blood of surgical patients with gastrointestinal cancer before infusion (t0), 3 days after infusion (t1), and 3 days after surgery (t2). Fluorescence was measured in FL3 (>670 nm), FL2 (585/42 nm), or FL1 (530/30 nm) channels by flow cytometry. Ten thousand events were evaluated per experiment. The values are presented as mean ± SEM of 12–13 samples. Data are expressed as relative decrease; *P < .05 for comparison before (t0) and after infusion or surgery (t1 or t2).

Figure 2. The effect of fish oil (FO) or medium/long-chain triglycerides (MCTs/LCTs) on cell viability (A), DNA fragmentation (B), phosphatidylserine externalization (C), and mitochondrial polarization (D) in neutrophils. Neutrophils were freshly isolated from the peripheral blood of surgical patients with gastrointestinal cancer before infusion (t0), 3 days after infusion (t1), and 3 days after surgery (t2). Fluorescence was measured in FL3 (>670 nm), FL2 (585/42 nm), or FL1 (530/30 nm) channels by flow cytometry. Ten thousand events were evaluated per experiment. The values are presented as mean ± SEM of 12–13 samples. Data are expressed as relative decrease; *P < .05 for comparison before (t0) and after infusion or surgery (t1 or t2).
colon and gastric cancer is supported by a recent meta-analysis of randomized controlled trials that provided science-based conclusions concerning the safety and efficacy of using FO–enriched PN to decrease the postoperative infection rate and length of stay in hospitals and intensive care units (ICUs) of patients undergoing general major abdominal surgery, including both gastric and colon cancer patients.23

Our sample size was based on our previous experience with studies evaluating the effect of parenteral lipid emulsions on leukocyte death in healthy volunteers, where we were able to observe changes in leukocyte death after the infusion of LE when at least 10 participants per treatment were evaluated.7,24

Although traditionally, FO emulsion has been infused as a supplement physically mixed with standard LEs (based on soybean or olive oil or rich in MCTs) in the amount of 10%–20% of total fat, its isolated use as a pharmacological agent has been considered to quickly modulate immune functions and possibly improve clinical outcomes. As recently demonstrated by Bahadori et al25 in patients with rheumatoid arthritis, the infusion of pure FO emulsion at 0.2 g of fat per kilogram of body weight is safe, well tolerated, and sufficient to improve clinical symptoms. To contribute to the scientific background in assessing this current issue, we explored the effect of the infusion of pure FO emulsion on leukocyte death.

According to our data, in surgical patients with gastrointestinal cancer, the infusion of MCT/LCT emulsion before surgical trauma did not modify neutrophil or lymphocyte death but may induce postoperative apoptosis of these leukocytes, as indicated by the increased proportions of cells with PS externalization or DNA fragmentation.

There is only 1 published study on leukocyte death by the infusion of MCT/LCT emulsion. Buenestado et al26 demonstrated that MCT/LCT emulsion does not induce apoptosis of stimulated neutrophils in vitro, as has been observed in the context of SO emulsion. Postoperative apoptosis mediated by MCT/LCT emulsion may occur through the influence of different factors, which depend on physiological interactions that are absent from simple in vitro models.

Major tissue injury response leads to the production of pro-inflammatory cytokines that may activate neutrophils and macrophages to release granular enzymes and produce ROS.12 Therefore, surgical stress can establish a cell-detrimental environment that is associated with organ dysfunction.13 Delogu et al27 demonstrated a significantly higher frequency of CD4+ and CD8+ T cell apoptosis at 24 hours after surgery. They also showed that the rate of CD8+ T cell apoptosis correlated with the rate of infectious complications manifested during the postoperative course. It is possible that the increase of postoperative lymphocyte and neutrophil apoptosis observed in patients infused with MCT/LCT emulsion arose from the modifications induced by surgical trauma, rather than from a pro-apoptotic effect of this LE.

Regarding the infusion of a pure FO emulsion, it resulted in a small proportional increase of lymphocytic death by necrosis, in relation to basal values (t0), but did not change cell apoptosis. Partly in agreement with our findings, the substitution of 20%

| Table 2. List of the Lymphocyte Genes Related to Cell Death Up- and Downregulated in Postoperative Surgical Patients With Gastrointestinal Cancer Treated Preoperatively with a Parenteral Lipid Emulsion Composed of a Physical Mixture of Soybean Oil and MCT/LCT or a Parenteral Lipid Emulsion of Pure FO |
|-----------------------------------------------|-----------------------------------------------|
| **Upregulation** | **FO** |
| MCT/LCT | FO |
| TNF receptor–associated factor 5 (TRAF5) | TNF receptor–associated factor 3 (TRAF3) |
| TNF receptor–associated factor 1 (TRAF1) | Colony-stimulating factor 1 (macrophage) (CSF1) |
| Fas ligand (TNF superfamily, member 6) (FASLG) | Colony-stimulating factor 2 (granulocyte-macrophage) (CSF2) |
| Avian v-mycremycmyelocytomatosis viral oncogene homolog (MYC) | BCL2-associated athanogene 4 (BAG4) |
| Connective tissue growth factor (CTGF) | Nonmetastatic cells 1 (NME1) protein (NM23A) |
| Nuclear gene encoding mitochondrial protein | |
| BCL2-like 1 (BCL2L1) | |
| BCL2-antagonist of cell death (BAD) | |
| Vascular endothelial growth factor (VEGF) | |
| Melanoma inhibitory activity (MIA) | |
| **Downregulation** | **FO** |
| MCT/LCT | FO |
| BH3 interacting domain death agonist transcript variant 1 (BID) | Bifunctional apoptosis regulator (BFAR) |
| CASP8 and FADD-like apoptosis regulator (CFLAR) | Growth arrest and DNA damage–inducible alpha (GADD45A) |
| CD14 antigen (CD14) | |
| | |
| FO, fish oil; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; TNF, tumor necrosis factor. |
and 40% of linoleic acid in 2 lipid emulsions by ω-3 PUFA also had no influence on apoptosis but was not associated with B and T lymphocyte necrosis. In our research, postoperative lymphocyte necrosis occurred at a very similar and slight extension compared with the preoperative period (5% vs 7%) and was probably not aggravated by surgical trauma.

In addition, we demonstrated that FO emulsion has a protective effect on postoperative lymphocyte apoptosis by substantially decreasing the proportion of these leukocytes with PS externalization. In addition, FO emulsion did not induce neutrophil death, even during the postoperative period, opposite to what was observed with MCT/LCT emulsion. Postoperative survival of neutrophils may represent an appropriated adaptive response to injury to eliminate invading pathogens and may be associated with the decreased postoperative rates of infectious complications observed in surgical patients treated with FO emulsion, compared with SO emulsion. In the present study, for both LEs studied, changes in leukocyte death were accompanied by alterations in the expression of genes related to cell death. Fatty acids have been increasingly recognized as major biological regulators. Many of the effects of fatty acids in cell biology and human health and disease are associated with their abilities, mainly n-3 PUFA, to regulate gene expression and subsequent downstream events. Possible mechanisms for these effects include the expression or processing of transcription factors, nuclear hormone receptors, and lipid second messengers. For instance, certain fatty acid–derived molecules have been shown to modulate inflammation by activating peroxisome proliferator-activated receptors (PPARs), transcription factors that, once activated, bind to their recognition sequences and regulate gene expression.

Our molecular data suggest that increased postoperative apoptosis of lymphocytes from patients preoperatively infused with MCT/LCT emulsion can be associated with the preactivation of proapoptotic pathways mediated by BAD, BCL-xL, and BCL2 (via mediation of outer mitochondrial membrane channel opening) and also TRAF5, TRAF1, and FASLG (via TNF family members and their receptors, with the activation of NF-κB). However, MCT/LCT emulsion was also associated with the inhibition of proapoptotic genes. BID is a death agonist that mediates mitochondrial damage induced by caspase-8 through cytochrome c release; the activation of Fas-associated protein with death domain (FADD) by CFLAR also regulates the death-inducing signaling complex (DISC), leading to apoptosis. This finding suggests a possible regulatory mechanism for apoptosis, and it is difficult to exclude the influence of other physiological factors related to surgical trauma on the higher postoperative lymphocyte apoptosis observed in MCT/LCT-treated patients. A more detailed protein study should contribute to a better understanding of these molecular findings.

Meanwhile, FO emulsion demonstrates a clear protective effect on postoperative lymphocyte death by downregulating proapoptotic and upregulating antiapoptotic genes. This emulsion downregulated BFAR and GADD45A genes. BFAR interacts with both the intrinsic and extrinsic apoptotic pathways, and GADD45A is a member of a group of genes that are increased after treatment with DNA-damaging agents and environmental stress by mediating activation of the p38/JNK pathway. In addition, FO emulsion also upregulated gene expression of TRAF3, CSF1, CSF2, and BAG4. TRAF3 serves as a negative regulator of the NF-κB pathway for TNF receptors, by blocking NF-κB activation via TRAF2/5. Members of the CSF family that play an important role in macrophage recruitment and activation (M-CSF) are synthesized through NF-κB and PPAR-γ regulation. Maianski et al suggested that CSF induces Akt protein phosphorylation, which inhibits proapoptotic proteins such as BCL-xl and BID. BAG4 is a member of the BAG1-related antiapoptotic protein family, which is associated with the death domain of TNF receptor type 1 (TNF-R1) and death receptor-3 (DR3) and thereby negatively regulates downstream cell death signaling.

The upregulation of antiapoptotic genes that involves PPAR and NF-κB is in accordance with previous reports that demonstrate the capacity of ω-3 fatty acids provided by FO to modulate gene expression by mediating these transcriptional factors. One proposed mechanism may include the displacement of lipid rafts, which are functional membrane microdomains related to the initiation and propagation of cell signaling. Therefore, the expression of TRAF3, CSF1, CSF2, and BAG4 genes may be associated with the protective effect of FO emulsion on postoperative lymphocyte PS externalization; however, a more detailed protein study should be developed to confirm this hypothesis. In summary, in patients with gastrointestinal cancer, the preoperative infusion of MCT/LCT is associated with postoperative lymphocyte and neutrophil apoptosis, whereas FO has a protective effect on postoperative lymphocyte apoptosis.

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