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What is This?
Partial Replacement of ω-6 Fatty Acids With Medium-Chain Triglycerides, but Not Olive Oil, Improves Colon Cytokine Response and Damage in Experimental Colitis

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Abstract

Background: Soybean oil is rich in ω-6 fatty acids, which are associated with higher incidence and more severe cases of inflammatory bowel diseases. The authors evaluated whether partial replacement of soybean oil by medium-chain triglycerides (MCTs) or olive oil influenced the incidence and severity of experimental ulcerative colitis by using different parenteral lipid emulsions (LEs). Methods: Wistar rats (n = 40) were randomized to receive parenteral infusion of the following LE: 100% soybean oil (SO), 50% MCT mixed with 50% soybean oil (MCT/SO), 80% olive oil mixed with 20% soybean oil (OO/SO), or saline (CC). After 72 hours of infusion, acetic acid experimental colitis was induced. After 24 hours, colon histology and cytokine expression were analyzed. Results: SO was not significantly associated with overall tissue damage. MCT/SO was not associated with necrosis (P < .005), whereas OO/SO had higher frequencies of ulcer and necrosis (P < .005). SO was associated with increased expression of interferon-γ (P = .005) and OO/SO with increased interleukin (IL)-6 and decreased tumor necrosis factor-α expression (P < .05). MCT/SO appeared to decrease IL-1 (P < .05) and increase IL-4 (P < .001) expression. Conclusions: Parenteral SO with high concentration of ω-6 fatty acids was not associated with greater tissue damage in experimental colitis. SO partial replacement with MCT/SO decreased the frequency of histological necrosis and favorably modulated cytokine expression in the colon; however, replacement with OO/SO had unfavorable effects. (JPEN J Parenter Enteral Nutr. 2012;36:442-448)

Keywords
medium-chain triglycerides, olive oil, soybean oil, colitis, lipid emulsion, parenteral nutrition

Clinical Relevancy Statement

Dietary management of ulcerative colitis may be an effective therapy for maintaining patients with this inflammatory bowel disease in the remission phase as much as possible. Dietary fatty acids are involved in several inflammatory processes; the ω-6 family is mainly proinflammatory. Parenteral fatty acid supplementation modulates immunological markers considerably faster than oral intake. Thus, we evaluated whether parenteral infusion of lipid emulsion in which the ω-6 content was partially replaced by ω-9 or medium-chain triglycerides would decrease the damage caused by experimental colitis.

Introduction

Ulcerative colitis (UC) and Crohn disease are the 2 main subtypes of inflammatory bowel diseases (IBD). UC affects mainly the colon, and inflammatory changes are limited to the mucosa. The etiology of IBD is poorly understood. Defects in mucosal barrier function and deregulated immune recognition of gut microbiota in genetically susceptible individuals are supported by epidemiological data. In addition, environmental factors may also be linked to IBD.

Increased ingestion of ω-6 polyunsaturated fatty acids (n-6 PUFA), mainly arachidonic acid or its precursor, linoleic acid, has been discussed as a potential contributor for IBD. Elevated levels of n-6 PUFA may contribute to creating a proinflammatory environment, whereas a reduction in n-6 PUFA intake could promote an environment less susceptible to inflammation.
The production of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)–1, and IL-6, can be stimulated by the leukotriene B4, the most powerful metabolite of arachidonic acid. In both forms of IBD, increased levels of these proinflammatory cytokines have been observed. This suggests that such mediators may play a role in determining the severity of the disease. Moreover, the clinical efficacy of therapies that target cytokines indicate that these mediators are deregulated and could contribute to aggravation of IBD and other chronic inflammatory conditions. Thus, reducing n-6 PUFA intake by introducing other fatty acids (FAs), primarily n-9 monounsaturated FA and medium-chain triglycerides (MCTs), may have beneficial immunomodulatory effects in UC.

We hypothesized that decreased concentrations of n-6 PUFA would be associated with changes in colonic cytokine expression and lower incidence and severity of UC. Parenteral FA supplementation has the advantage of rapidly modulating immunological markers (48–72 hours), and it avoids the digestive and absorptive FA losses that usually occur with oral and enteral intake.

We tested our hypothesis by studying the effect of commercially available parenteral lipid emulsions (LEs) with different concentrations of n-6 PUFA on the incidence and severity of experimentally induced UC in rats. A soybean oil–based LE (a rich source of n-6 PUFA) was compared to 2 other LEs that are rich in either MCTs or olive oil; both of these LE contain approximately 50% less n-6 PUFA than soybean oil LE.

Materials and Methods

Animals

The study protocol was approved by the Research Ethical Committee (CAPPesq), School of Medicine, University of São Paulo (FMUSP), São Paulo, Brazil. Forty adult male Wistar rats (250–300 g) were obtained from the Vivarium Center (Biotério Central) of the School of Medicine, University of São Paulo. Prior to the experimental procedures, animals were adapted for 5 days at controlled room temperature (22 ± 2°C), under day-night light cycles, in metabolic cages, with free access to standard rodent chow and water. In sequence, 2 weeks before the experimental procedures, the animals were treated with vermiculfe praziquantel (25 mg/kg body weight) and ivermectin/pyrantel (2.0 µg/kg body weight), both from Merck Sharp & Dohme (Haar, Germany).

Parenteral Access

Animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg of body weight), and parenteral access was achieved by jugular vein cannulation, according to a standard technique, followed by a connection to a swivel apparatus to allow the animals free mobility. All rats were then housed in metabolic cages. All intravenous treatments were delivered with a peristaltic pump (0.5 mL/h rate) for 72 hours.

The animals were randomized for intravenous infusions with 1 of 4 regimens. The control group (CC) received 0.9% saline solution; the soybean oil group (SO) received Lipovenoes 20% (Fresenius-Kabi, Bad Homburg, Germany), which comprised 100% soybean oil, rich in n-6 PUFA; the medium-chain triglycerides group (MCT/SO) received Lipofundin 20% (B. Braun Melsungen, Melsungen, Germany), a physical mixture of 50% MCTs and 50% soybean oil; and the olive oil group (OO/SO) received ClinOleic 20% (Baxter, Maurepas, France), which comprised 80% olive oil, rich in n-9 monounsaturated FA, and 20% soybean oil. The FA compositions of the different LEs are listed in Table 1. All LE regimens were delivered at doses of 8–9 g of fat/kg body weight. The CC received a standard oral diet (AIN-93M), and the LE-treated groups received lipid-free oral diets.

Experimental Colitis

After 72 hours of intravenous infusion, experimental colitis was induced in all of the animals in this study by an intrarectal administration of 0.5 mL of 10% acetic acid solution, as described elsewhere. The animals were killed 24 hours after the colitis procedure. Before they were killed, the rats were weighed and anesthetized. Laparotomy was performed for complete colon resection and sample collection. The colon specimens were washed with saline solution and dissected longitudinally for macroscopic analysis. Then, specimens were placed in a 10% formaldehyde buffer. After dehydration and standard processing, the samples were paraffin-embedded in individual blocks for further histological, cytokine expression, and immunohistochemistry analyses.

Macroscopic and Histological Analyses

The specimens were evaluated macroscopically for the presence of hyperemia, scaling, mucus, ulcer, edema, ischemia, and tissue necrosis.

Specimens were cut into 3.0-µm sections and stained with hematoxylin-eosin. Sections were histologically analyzed for the presence of reactive lymphoid hyperplasia, extensive ulceration, extensive ulceration, inflammatory infiltrates in the submucosa and muscle, and transmural necrosis. All of the evaluations were performed with an optical microscope equipped with 200–400x objectives (standard objectives; Nikon, Tokyo, Japan; and Zeiss, Jena, Germany) by 2 independent observers who were blinded to the experimental groups. Measurements from 5 randomized, high-power optical fields were averaged for each rat. Disagreements regarding observations between the 2 investigators (eg, presence vs absence of necrosis) were reviewed simultaneously and a consensus was reached.
Cytokine Expression

We determined the expression of cytokines IL-1, IL-4, IL-6, TNF-α, and interferon (IFN)–γ with immunohistochemical methods that were standardized and described previously.8 Briefly, after deparaffinization, 3.0-µm histological sections of the colon were incubated overnight with primary cytokine-specific antibodies. Antibodies were diluted with phosphate-buffered saline (PBS) 1:300 for anti-rIL-1β and 1:30 for anti-rIL-4, anti-rIL-6, anti-rIFN-γ, and anti-rTNF-α (all from R&D Systems, Minneapolis, Minnesota). All of the test reactions (tissue with primary antibody) were run in parallel with negative controls (tissue and reaction buffer with no primary antibody).

Two observers, also blinded to the experimental groups, counted positive cells in 10 different fields (400× magnification) with high concentrations of positively identified inflammatory cells (hot spots). Stromal and epithelial cells were not counted. A mean of the preliminary 10 results obtained by each observer for each rat was obtained and then a new mean was calculated considering each of these 2 obtained means. Cases with severe disagreement were reviewed simultaneously and a diagnostic consensus was reached.

Statistical Analysis

Normal distributions of variables were assessed with the Kolmogorov-Smirnov test. Data are expressed as the mean ± standard deviation. For macroscopic and histological analyses, the Monte Carlo $\chi^2$ exact test was used to assess differences among groups. For cytokine evaluation, comparisons between groups were carried out with 1-way analysis of variance (ANOVA) and post hoc comparisons with Tukey’s test; when normality or equal variance failed, the Kruskal-Wallis and Dunn post hoc tests were used. Fisher’s exact test was performed to assess correlations between cytokine expression and macroscopic alterations. P values <.05 were considered statistically significant. Statistical analyses were carried out with SPSS 10.0 for Windows (SPSS, Inc, an IBM Company, Chicago, Illinois).

Results

Body Weight and Infusion Volume

Regardless of nutrition treatment, all of the rats lost body weight (8.86 ± 3.16 g on average), with no statistical differences among groups ($P = .175$). The animals in the OO/SO group exhibited less food intake (16.2 ± 4.19 g) than animals from the other groups ($P < .05$). There were no statistical differences in the infused volumes of LE or saline among the 4 groups.

Macroscopic Alterations

Macroscopic alterations were observed in the colons of all animals subjected to colitis (Table 2). Necrosis was the only macroscopic alteration observed that was statistically different.
Medium-Chain Triglycerides in Experimental Colitis

Mucosa different between groups (Figure 1). and submucosal inflammatory infiltrates were not significantly were observed in the OO/SO group compared to the other groups. Colon

Among the several experimental models available for UC research, we decided to use acetic acid–induced UC in rats to evaluate whether reduced infusion of potentially proinflammatory n-6 PUFA would affect tissue damage and cytokine expression in the colon. After injury, cytokine expression may peak at different times, but based on the results of previous studies, reasonable changes should be detectable after 24 hours. In a previous study performed by our laboratory, after 24 hours of acetic acid–induced colitis, we observed a significant increase in IL-1, IFN-γ, and IL-6 expression, as well as a significant increase in the frequency of colonic necrosis compared to noccolitis controls. In addition, the increase in both IFN-γ and IL-6 was significantly associated with the presence of histological colonic necrosis in the group with colitis.8

We compared commercial parenteral soybean LE with a high concentration of n-6 PUFA (about 50%) to 2 other LEs with lower concentrations of these FAs (about 20%-25%), both of which claim to have a neutral impact on the immune response. The amount of fat supplementation in the experimental groups was estimated with the goal of providing 30%-40% of nonprotein calories, similar to the percentages used in studies by other authors,17,20 and in accordance with the recommendations of the European Society for Clinical Nutrition and Metabolism (ESPEN).21

We found that infusion of parenteral LE modified the incidence and severity of rat colonic tissue damage and cytokine profile expression 24 hours after insult, apparently independent of reductions in n-6 PUFA concentrations. Soybean oil–based LE increased proinflammatory IFN-γ, but rats in this group had significantly less frequent macroscopic colonic necrosis when compared to rats infused with OO/SO LE. This finding is supported by a previous study in which mice were fed an arachidonic acid–enriched diet followed by induction of colitis by dextran sodium sulfate.22 The mice in this study had increased concentrations of colonic arachidonic acid, but this was not associated with increased colonic inflammation.22

In our experiment, 24 hours after colitis induction, cytokine response and histological damages were different among the groups infused with diluted n-6 PUFA LE. The MCT/SO infusion favorably modulated colonic cytokine expression by reducing proinflammatory IL-1 and increasing anti-inflammatory IL-4, and there was no histological presence of necrosis. By contrast, rats that received infusions of OO/SO, which is rich in n-9 monounsaturated FA (MUFA), had decreased TNF-α levels but increased proinflammatory cytokine IL-6 levels. Histological analysis identified higher frequencies of ulcer and necrosis in the OO/SO group compared to all other rat groups.

The 3 LEs that we used in this study are not composed exclusively of FAs. In addition to egg phosphatides and glycerol, they also contain substantial amounts of the antioxidant α-tocopherol, which has anti-inflammatory properties that could influence our findings. However, olive oil–based LE has a high concentration of α-tocopherol (32 mg/L from olive oil; data provided by the manufacturer) and did not have significant anti-inflammatory effects. Therefore, we propose that the

| Table 2. Colon and Mucosa Macroscopic Alteration in Rats Observed After 24 Hours of Colitis Induction |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | CC              | SO              | MCT/SO          | OO/SO           |
| Colon           |                 |                 |                 |                 |
| Hyperemia       | 2               | 5               | 4               | 3               |
| Edema           | 2               | 0               | 0               | 2               |
| Ischemia        | 0               | 2               | 2               | 3               |
| Necrosis        | 8               | 4               | 5               | 4               |
| Mucosa          |                 |                 |                 |                 |
| Hyperemia       | 1               | 5               | 4               | 1               |
| Edema           | 0               | 2               | 1               | 0               |
| Ischemia        | 0               | 0               | 0               | 0               |
| Necrosis        | 7               | 2               | 7               | 8*              |
| Ulcer           | 9               | 6               | 5               | 6               |
| Scaling         | 0               | 4               | 3               | 1               |
| Mucus           | 0               | 0               | 0               | 0               |

The data are expressed in absolute numbers of positive events, mean value/group. Control rats (CC) received saline infusions, and test rats received lipid emulsion infusions that primarily contained soybean oil (SO), medium-chain triglycerides (MCT/SO), or olive oil (OO/SO). Significant changes are indicated as *P = .0235 vs SO.

Histological Analysis

Higher frequencies of ulcers (P < .001) and necrosis (P = .003) were observed in the OO/SO group compared to the other groups. Necrosis was absent in the MCT/SO group. Hyperplasia, muscle, and submucosal inflammatory infiltrates were not significantly different between groups (Figure 1).

Cytokine Expression

The SO group had higher IFN-γ expression in the colon compared to all other groups (P = .005). The MCT/SO group had significantly lower IL-1 expression (P < .05) and higher IL-4 expression (P < .001) compared to the SO and OO/SO groups.

The OO/SO group had significantly higher IL-6 production (P < .05) compared to all other groups and lower TNF-α expression compared to the CC group (P = .042). Cytokine expression results are listed in Table 3.

An analysis of the correlations between cytokine expression and macroscopic alterations revealed that high levels of IL-6 and IFN-γ were positively associated with the presence of histological necrosis in the CC group (P = .005).

Discussion

Among the several experimental models available for UC research, we decided to use acetic acid–induced UC in rats to

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immunomodulatory properties of these LEs are associated with their FA composition rather than due to other ingredients. If this is so, then the varied effects of MCT/SO and OO/SO were likely a result of the type of FA used to dilute the soybean oil rather than due solely to the reduced amounts of infused n-6 PUFA.

Traditionally, MCTs have been considered only as an energy source, but recent data indicate that MCTs may also have immunomodulatory effects. We and others have reported that MCT-containing LE can modulate leukocyte functions in vitro, in vivo, and ex vivo; these functions include monocyte recruitment stimulation, increased numbers of phagocytosing macrophages, and increased respiratory burst with the production of reactive oxygen species by polymorphonuclear leukocytes. In addition, mice fed with MCT-enriched diets have lower plasma proinflammatory (TNF-α and IL-1β) and higher plasma anti-inflammatory (IL-10) responses to endotoxin compared to n-6 PUFA-, n-9 MUFA-, and n-3 PUFA-enriched diets.

In another study about the effects of MCT in colitis, in a model of spontaneous intestinal inflammation in IL-10-deficient mice, partial replacement of dietary n-6 PUFA with MCT decreased the incidence of colitis. Parenteral infusion of MCT-based LE in rats with induced colitis was also associated with protection of the mucosa and reduced intestinal atrophy.

n-9 MUFAs have been associated with neutral effects on immune and inflammatory functions. In the present study, we found that OO/SO LE infusion did not have a neutral effect and did not prevent colon damage. Instead, it increased the local expression of IL-6. High serum concentrations of inflammatory markers, such as IL-6 and C-reactive protein (CRP), were previously associated with anorexia. Thus, the significantly elevated levels of IL-6 that we observed in the

Figure 1. Histological parameters observed after 24 hours of colitis induction. Control rats (CC) received saline infusion, and test rats received lipid emulsion infusion that primarily contained soybean oil (SO), medium-chain triglycerides (MCTs/SO), or olive oil (OO/SO). The data are expressed in absolute numbers, mean value/group and SD. Significant changes are indicated as follows: *P < .001, OO/SO > CC, SO, and MCT/SO; #P < .005, OO/SO > CC, SO, and MCT/SO. Necrosis was absent in MCT/SO (#P < .005 when compared to other groups).

Table 3. Cytokine Expression in Rats After 24 Hours of Colitis Induction

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1</th>
<th>IL-4</th>
<th>IL-6</th>
<th>IFN-γ</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>9.29 ± 4.26</td>
<td>7.92 ± 3.90</td>
<td>8.71 ± 2.20</td>
<td>5.51 ± 2.06</td>
<td>16.55 ± 7.21</td>
</tr>
<tr>
<td>SO</td>
<td>14.14 ± 8.38</td>
<td>4.59 ± 0.98</td>
<td>7.46 ± 0.54</td>
<td>9.08 ± 2.99²</td>
<td>16.29 ± 3.07</td>
</tr>
<tr>
<td>MCT/SO</td>
<td>4.56 ± 3.43*</td>
<td>11.15 ± 3.37*</td>
<td>7.32 ± 2.20</td>
<td>4.87 ± 0.95</td>
<td>15.66 ± 4.71</td>
</tr>
<tr>
<td>OO/SO</td>
<td>15.81 ± 11.18</td>
<td>5.86 ± 1.98</td>
<td>11.15 ± 1.07⁶</td>
<td>6.19 ± 2.39</td>
<td>10.19 ± 4.70⁶</td>
</tr>
</tbody>
</table>

The data are expressed in number of positive cells, mean ± standard deviation. IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α. Control rats (CC) received saline infusions, and test rats received lipid emulsion infusions that primarily contained soybean oil (SO), medium-chain triglycerides (MCTs), or olive oil (OO). Significant changes are indicated as follows: *P < .05 vs SO and OO/SO; ²P < .05 vs all groups; and ⁶P < .05 vs CC.
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OO/SO group could explain, in part, why this group ingested less oral diet than the other groups.

In contrast to our findings, a previous study in mice with dextran sodium sulfate–induced colitis found that oral treatment with extra-virgin olive oil led to lower levels of the colonic proinflammatory cytokine cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) expression was significantly lower than in mice treated with sunflower oil.37 The differences between these data and ours can be explained by the differences in the experimental models used, such as the method of colitis induction and the route by which animals were fed.

To the best of our knowledge, this study was the first to evaluate the effects of olive oil–based LE infusion on colitis. Olive oil has been used as a placebo in studies that assessed other FAs in colitis treatments, mainly n-3 PUFA.1,38,39 Our findings suggest that olive oil should not be used as a placebo in inflammatory conditions because it may not be innocuous. Nonneffe effects of n-9 MUFA were observed by Gassull et al40 in humans. In their randomized, double-blind study, the remission rate of active Crohn disease was significantly lower in patients after 4 weeks of treatment with an enteral diet rich in n-9 MUFA (27%) compared to those treated with an enteral diet rich in n-6 PUFA (63%).40 However, it should be emphasized that human inflammatory bowel diseases are physiopathologically different from chemically induced acetic colitis, which precludes any generalizations to humans based on our results.

One possible mechanism that could partially explain the increased IL-6 production and histological necrosis observed with OO/SO-based LE infusion is a possible change in the composition of lipid rafts on the surface of leukocytes of the gut-associated lymphoid tissue (GALT) system and/or colonocytes. Lipid rafts are membrane microdomains that are responsible for modulating receptor-mediated signal transduction, thus influencing cell activation. Diet and plasma-derived nutrients may modulate inflammation by altering lipid raft–associated cellular activation. For example, ω-3 FAs have been shown to activate leukocyte chemotaxis and resident-macrophages’ phagocytosis in rats.14

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References


