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Oral omega-3 fatty acids promote resolution in chemical peritonitis

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ABSTRACT

Background: Recent studies suggest that purified omega-3 fatty acids may attenuate acute inflammation and hasten the transition to healing. In this study, we tested the hypothesis that pretreatment with omega-3–rich fish oil (FO) would promote resolution of peritoneal inflammation through production of specific lipid mediators.

Methods: C57/BL6 mice were given a daily 200-μL oral gavage of saline (CTL) or FO (1.0-1.5 g/kg/d docosahexaenoic acid and 1.3-2.0 g/kg/d eicosapentaenoic acid) for 7 d before chemical peritonitis was induced with thioglycollate. Peritoneal lavage fluid was collected before induction and at days 2 and 4 after peritonitis onset. Prostaglandin E2 (PGE2), Leukotriene B4 (LTB4), Resolvin D1 (RvD1), and the composition of immune cell populations were examined in peritoneal lavage exudates. Cells harvested from the peritoneum were assessed for macrophage differentiation markers, phagocytosis, and lipopolysaccharide-induced cytokine secretion profiles (interleukin [IL]-6, IL-10, IL-1β, TNFα).

Results: The ratio of RvD1 to pro-inflammatory PGE2 and LTB4 was increased in the peritoneal cavity of FO-supplemented animals. FO induced a decrease in the number of monocytes in the lavage fluid, with no change in the number of macrophages, neutrophils, or lymphocytes. Macrophage phagocytosis and M1/M2 messenger RNA markers were unchanged by FO with the exception of decreased PPARγ expression. FO increased *ex vivo* TNFα secretion after stimulation with lipopolysaccharide.

Conclusions: Our findings provide evidence that nutraceutically relevant doses of FO supplements given before and during chemical peritonitis shift the balance of lipid mediators towards a proresolution, anti-inflammatory state without drastically altering the number or phenotype of local innate immune cell populations.

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Introduction

Resolution of local and systemic inflammation is critical in the process of recovery from peritonitis. Once thought to be a passive process involving depletion of pro-inflammatory mediators, resolution is now known to be an active, cell-mediated progression involving the actions of specific lipids and proteins as drivers of the resolving response. Novel discoveries[1-3] have illuminated the second half of this bimodal response to immune challenge, which is dictated in part by bioactive lipid mediators with varied functions. As reviewed elsewhere,[4] proresolution lipid mediators—resolvins, maresins, and protectins—appear during the first phase of inflammation and are capable of inducing the resolution program, which includes the eventual deceleration of recruitment of neutrophils and acceleration of recruitment of macrophages to inflamed spaces.[3,5] In the resolution phase, the profile of the macrophage population is switched from M1 (pro-inflammation) to M2 (proresolution),[6] which includes decreased production and/or downregulation of pro-inflammatory cytokines[7,8] and alterations in immune cell shape and morphology consistent with the scavenging of nonviable cells and material, a process known as efferocytosis.[9] It has been postulated that altered ratios of proresolution to pro-inflammation lipids (prostaglandins, leukotrienes) may effectively dictate the character of an inflammatory response by shifting the balance of pro- and anti-inflammatory cytokines and chemokines.[10]

Endogenous production of proresolving lipid mediators depends on availability of specific precursors in the omega-3 (n-3) fatty acid class, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Thus, it has been hypothesized that resolution of certain states of chronic inflammation might be interrupted by treatment with these compounds, either in purified form or in the form of fish oil (FO) supplements. As the FO industry has grown to become a nearly $2 billion market and recent surveys have shown that one-third of Americans taking nutritional supplements may be using an FO supplement,[11] the need exists to carefully examine how omega-3 administration may affect medical care, especially in relation to invasive procedures. Indeed, the benefits of purified and dietary supplement forms have been observed in some settings associated with the surgical patient. Recent meta-analyses show that enteral immunonutrition regimes, which include omega-3 supplements, may optimize recovery of patients who undergo elective surgery for gastrointestinal cancer, especially when delivered in the perioperative period,[12,13] whereas some parenteral models of omega-3 supplementation have also shown beneficial effects.[14,15] In murine models, a similar mixed picture exists, but in congruence with human models, a majority of studies providing FO supplementation over periods of 5 d-12 wk display downstream resolution–favoring changes in messenger RNA (mRNA) expression of classical M1/M2 macrophage markers of maturation,[16] inflammatory cytokine and lipid profile,[17-19] efferocytosis,[20] and immune cell oxidative potency,[6] with additional reports indicating that oral FO takes 24-72 h to induce beneficial metabolic effects.[7]

It remains unclear what sorts of benefits would be observed in settings of more acute inflammation such as recovery from surgery.[21,22] Critical concerns in design of such studies include the demonstration that FO supplementation actually results in increased production of proresolution lipid mediators and that they are available in the inflamed space. Also important is the need to characterize changes in profiles of pro- and anti-inflammation cytokines resulting from such supplementation.[23-25] Finally, it remains unclear whether dietary supplements can elicit the phenotypic switch to resolution with efficacy similar to that of purified forms of DHA/EPA but with less toxicity.

In this study, we used a mouse model of sterile chemical peritonitis to characterize the local transition from acute inflammation to resolution in response to dietary FO supplements that may be obtained commercially. Thioglycollate is a relatively mild chemical irritant that provides a reproducible and consistent approach to study cascades of inflammation and resolution in the mouse and rat analogous to those in chemical and mechanical peritonitis conditions such as gastric acid leak, abdominal trauma, and surgical bowel irritation, without resulting in the presence of confounding polymicrobial infection.[26] Peritoneal injection of thioglycollate has been used as a low mortality, low systemic sequelae method to recruit and subsequently harvest inflammation-driving resident macrophages for the purposes of *in vitro* and *ex vivo* study and captures cells transitioning between acute inflammation and active resolution (3-4 d after injection) with peak inflammatory response in the first 1-2 d and complete clearance by day 7-8.[27] Using this model, we find that relevant doses of oral FO favor production and local appearance of resolution-phase lipid mediators but may yet be unable to elicit the dramatic changes in cell number and cytokine production that would be required to affect a clear benefit in accelerating recovery from an acute sterile inflammatory insult.

Materials and methods

Animals and experimental design

All animal procedures were approved and conducted in accordance with the Institutional Animal Care and Use Committee of Penn State Milton S. Hershey College of Medicine. Male C57BL/6 mice were obtained commercially (Taconic, Hudson, NY) and individually housed in polycarbonate cages with a 12-h light/dark cycle. Solely male mice were used, as recent reports have displayed differences in leukotriene biosynthesis between genders after chemical peritonitis.[28] Animals were fed *ad libitum* with the commercial AIN-93M (MP Biomedicals, Santa Ana, CA) diet containing 4% soybean oil for 28 d starting at 10-12 wk of age. On days 21 through 28, mice were given 200-μL daily oral gavages of PBS as control (CTL) or Marinol C-38 (FO; Stepan, Maywood, NJ), a proprietary FO blend containing 17% DHA and 24% EPA (approximately 1.0-1.5 g/kg/d DHA and 1.3-2.0 g/kg/d EPA). At day 28, mice from each group were either euthanized to establish baseline infiltrative and secretory characteristics of