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The molecular impact of omega 3 fatty acids on hepatic pro-inflammatory cytokine signaling

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ABSTRACT

Purpose: Parenteral nutrition associated liver disease (PNALD) develops in a subset of children receiving parenteral nutrition for intestinal failure. Omegaven™ is an omega-3 fatty acid (Ω3FA) lipid emulsion high in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that can lessen PNALD. Inflammatory cytokines (IL-1, TNF-α, TGF-β) are elevated in PNALD and can decrease paraoxonase 1 protein expression (PON1). We sought to determine the effect of Omegaven™, EPA, and DHA on inflammatory cytokines TNF-α, IL-1, and TGF-β via ERK1/2 and p-Smad2/3 signaling pathways as well as the changes in intracellular PON1 protein expression as a potential mechanism explaining the protective effects of Omegaven™ and Ω3FA.
Methods: HepG2 cells were cultured with each cytokine and Omegaven™, or EPA and DHA, or Intralipid™. P-Smad2/3 and PON1 protein levels were measured by Western blotting. ERK1/2 signaling was studied using homogenous time resolved fluorescence.
Results: Omegaven™ decreased TGF-β mediated Smad2/3 signaling by 30% (70% of control ± 12, $p < 0.03$). Omegaven™ decreased IL-1 and TNF-α mediated ERK1/2 signaling (0.49 fold ± 0.09, $p < 0.05$ and 0.22 ± 0.05, $p < 0.05$) compared to control.
Conclusion: Our results describe potential mechanisms by which Omegaven™ and Ω3FA can be hepatoprotective in the setting of PNALD by abating inflammatory cytokine signaling.

Short bowel syndrome (SBS) is a form of intestinal failure that results from inadequate length of small intestine to fully digest and absorb nutrients and calories [1]. Parenteral nutrition associated liver disease (PNALD) occurs in 40%–60% of infants and children receiving prolonged courses of life-saving parenteral nutrition (PN) for the treatment of conditions causing intestinal failure, including SBS [2]. The disease spectrum of PNALD includes steatosis, cholestasis, fibrosis, and ultimately cirrhosis [3]. The pathogenesis driving PNALD remains incompletely understood. The current FDA approved lipid emulsion, Intralipid®, is a 100% soybean oil-based lipid emulsion that contains predominantly omega-6 fatty acids, which may contribute to the progression of PNALD [3]. Treatment options for PNALD include stopping the TPN, decreasing lipid calories, reintroducing enteral feeds, and/or switching to other lipid sources such as Omegaven™ [3]. Omegaven™ is a fish oil-based lipid emulsion that is high in omega-3 fatty acids (Ω3FA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) [4,5]. Several published case reports suggest Omegaven™ has lessened and even reversed some stages of PNALD, however, the mechanisms of this effect are unknown [4,5].

In the early stages of PNALD, the liver undergoes inflammatory changes. Inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α), have been shown to be elevated in PNALD and to directly contribute to liver injury [6–8]. Studies have also demonstrated that the aforementioned inflammatory pathways can activate the transforming growth factor beta (TGF-β) pathway, which may contribute to further hepatocellular damage and fibrosis [8,9]. Paraoxonase 1 (PON1) is an antioxidant protein that is mainly synthesized and released by the liver [10]. It possesses lactonase activity and hydrolyzes lipid peroxides [11]. Systemically, it circulates in plasma bound to high density lipoprotein (HDL) and is thought to play an important anti-inflammatory role [10,11]. Previous studies demonstrate that the inflammatory cytokines IL-1 and TNF-α decrease PON1 mRNA levels but these studies did not include an analysis of PON1 protein expression [12]. To date, there is a paucity of data relating inflammatory cytokine pathways to PON1 protein expression and the role that Ω3FA might play in these pathways and on PON1. The aim of this study is to further understand the protective effects of Omegaven™, including its key components, Ω3FA, DHA, and EPA on IL-1, TNF-α, and TGF-β-mediated signaling pathways and liver damage in an *in vitro* model of PNALD.

We hypothesize that the damaging effects of cytokines will result in a decrease in PON1 protein expression and that this effect will be prevented by Omegaven™.

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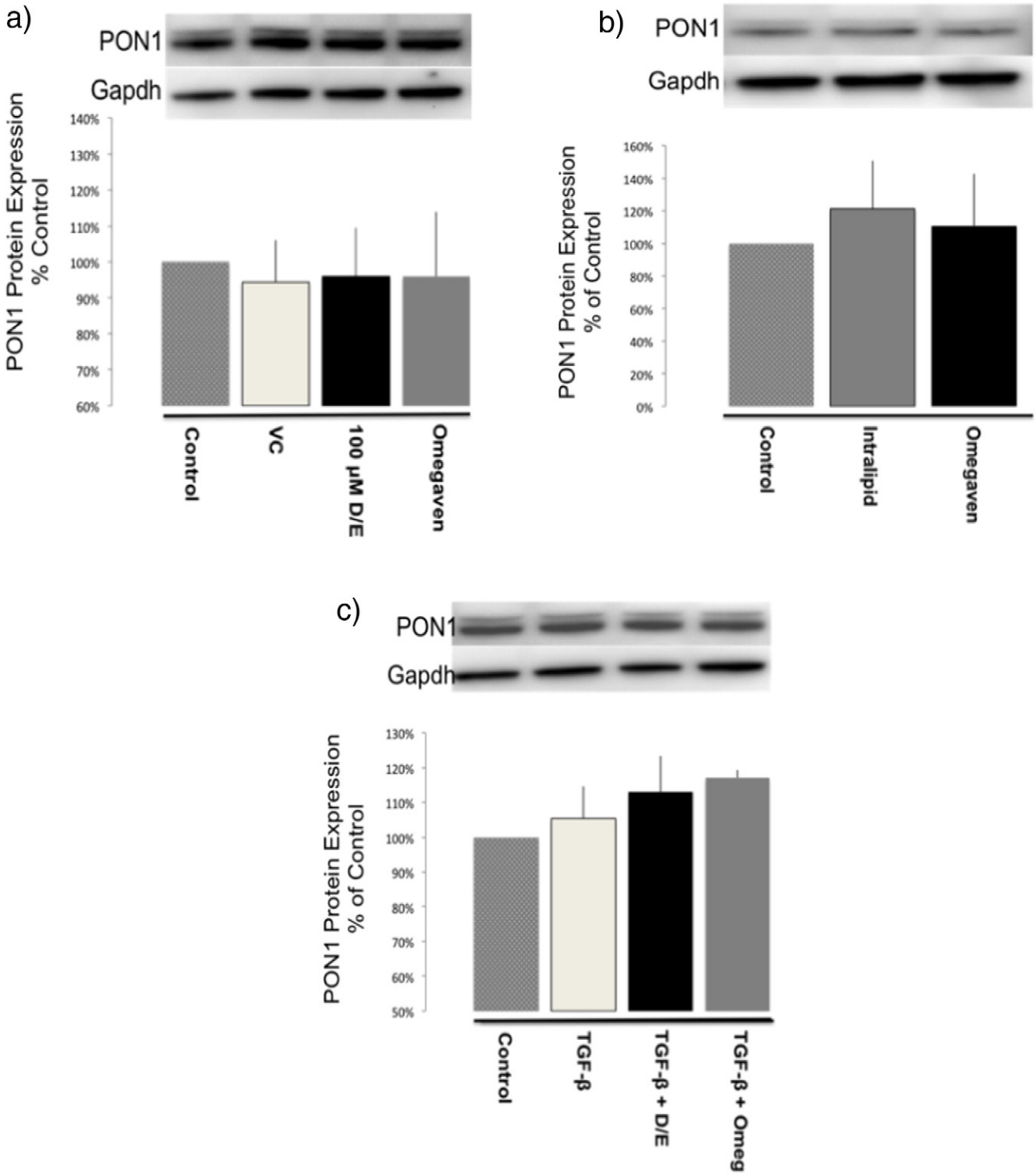

Fig. 1. (a) Bar graph and representative Western blot showing no statistical difference in PON1 protein expression when treated with controls, Ω3FA, or Omegaven™. (b) Bar graph and Western blot showing no statistically significant difference between treatment and control, Intralipid™, or Omegaven™ on PON1 protein expression. (c) Bar graph and representative Western blot showing no statistical effect of TGF-β on PON1 protein expression. VC = Vehicle control, ethanol. D/E = 100 μM DHA + EPA. n = 3.

1. Methods

1.1. Cell culture

Human hepatic HepG2 cells were cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated FBS in 100 mm plastic plates and kept between 50% to 80% confluence. Cells were plated in six well plates at 3×10^6 per well and allowed to adhere overnight. Cells were then treated with DMEM supplemented with either 20% Omegaven™ (Fresenius SE & Co. KgaA, Bad Homburg, Germany) 20% Intralipid® (Fresenius SE & Co. KgaA, Bad Homburg, Germany), or a 100 μM mixture of DHA and EPA (Cayman Chemical, Ann Arbor, MI) in DMEM for 1 h, followed by the addition of either TNF-α (50 ng/mL) or IL-1 (50 ng/mL) for another 16 h. TGF-β was also used (50 ng/mL) and treated cells were incubated overnight for PON1 analysis or 60 min for p-Smad2/3 analysis as described previously [13]. Cells were harvested, lysed, and PON1 and p-Smad2/3 expression was measured by Western blot analysis.