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Reduction of hepatocellular injury after common bile duct ligation using omega-3 fatty acids

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Abstract

Purpose: Bile duct obstruction and subsequent cholestasis produces hepatocellular injury and an inflammatory response. Fatty acid constitution of cell membranes plays a major role in the inflammatory cascade. Omega-3 fatty acids are antiinflammatory. We proposed that omega-3 fatty acid supplementation would reduce hepatocellular damage and cell death in a model of murine common bile duct ligation.

Methods: Mice underwent bile duct ligation and were administered either control soy diet (omega-6) or Menhaden diet (omega-3), and parameters of liver injury were measured at postoperative days 1, 4, and 8. Serum was analyzed for liver function tests. Liver tissue was scored for histologic necrosis and inflammation, and apoptosis was qualitatively measured.

Results: At day 8, comparing control and Menhaden, liver function tests were not significantly different. The H&E slides were analyzed and scored. At day 4, the mean necrosis scores for the Menhaden-fed group was 0.01 ± 0.028 and 0.46 ± 0.108 for the soy-fed group ($P = .001$) and at day 8, 0.420 ± 0.107 and 1.22 ± 0.132 ($P < .001$). The mean portal inflammation score for day 4 Menhaden-fed and soy-fed mice was 1.40 ± 0.245 for both groups ($P = 1.00$) and for day 8, 1.80 ± 0.200 and 2.80 ± 0.200 ($P = .008$). At day 1, the median terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling scores of the Menhaden vs soy group were 6.0 and 0.0 ($P < .001$); day 4, 24.0 and 3.0 ($P < .001$); and day 8, 0.0 and 3.0 ($P < .001$), respectively.

Conclusion: Although there appears to be a trend toward biochemical protection and a marked reduction of necrosis and inflammation, there was no significant liver function test difference between control and Menhaden groups. Considering our data of blunted histologic hepatotoxicity with omega-3 fatty acid supplementation, we hypothesize that this may be a method of reducing long-term complications of liver injury secondary to diseases of cholestasis such as biliary atresia, namely fibrosis and cirrhosis.

Bile duct obstruction and subsequent cholestasis produces hepatocellular injury and an inflammatory response. Varying degrees of these processes occur in patients with chronic liver disease. Cholestasis results in poor bile secretion and

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subsequently, poor lipid digestion and absorption of fat-soluble vitamins. In addition, intraluminal flora is altered with increased bacterial translocation, portal bacteremia, and endotoxemia [1,2]. The accumulation of toxic bile salts within the liver produces apoptosis and necrosis of hepatocytes. In turn, cell turnover may lead to scarring, fibrosis, and eventually, cirrhosis and liver failure [3]. Many liver diseases, such as biliary atresia, primary biliary cirrhosis, hepatitis B, alcoholic hepatitis, and autoimmune hepatitis, demonstrate a cholestatic pathophysiology [4-7].

Hepatocellular damage from common bile duct ligation occurs via 3 different mechanisms as follows: tumor necrosis factor α (TNF-α), Fas ligand (FasL), and oncotic necrosis. Tumor necrosis factor α is an inflammatory cytokine that has been implicated as a mediator of liver injury and apoptosis. Fas ligand is a membrane protein that is also a mediator of apoptosis via trimerization with the Fas receptor (FasR) and subsequent activation of caspase-8, which appears to be involved in cholestatic liver injury (ie, common bile duct ligation) [8]. Fas ligand has also been implicated in cirrhosis, secondary to cholestasis and viral infection [9]. Oncotic necrosis is characterized by cellular swelling and rupture in response to adenosine triphosphate (ATP) depletion [10].

Our previous work with parental nutrition–associated liver disease (PNALD) encouraged us to examine the possibility that omega-3 fatty acids may be effective against liver damage secondary to cholestasis [11-14]. Parenteral nutrition–associated liver disease is characterized by a spectrum of fatty liver disease, inflammation, bile duct proliferation, fibrosis, and cirrhosis. Biochemically, the disease is in part because of TNF-α–mediated inflammation and FasL-mediated apoptosis in animal models [15,16]. In our initial studies, we hypothesized that the development of PNALD may be dependent on the type of fat administered (soybean oil–based vs fish oil–based) and that omega-3 fatty acids would prevent or reduce de novo lipogenesis and subsequent liver injury. In this model, mice were treated with oral PN for 19 days. This energy load was similar to the established dietary energy needs of the mouse [17]. Experimental groups were supplemented with Intralipid 20% (Baxter, Deerfield, Ill), which is primarily plant-derived (omega-6), and fish oil (omega-3). Mice that received PN without fat supplementation developed fatty liver injury. Mice receiving intravenous Intralipid had even more severe liver changes. Both groups of animals developed marked hepatic steatosis with macrovesicular fatty infiltration and significant elevations in spectroscopic liver fat content and serum transaminase levels. However, animals receiving fish oil had completely normal livers on histologic examination, and spectroscopy revealed normal liver fat content. In addition, there was no fatty acid deficiency in either group as determined by serum fatty acid analysis.

A hepatoprotective effect of omega-3 fatty acids has been demonstrated in a study by Schmocker et al [18], in which Fat1 mice (genetically engineered to endogenously convert omega-6 fatty acids to omega-3 fatty acids) were subjected to the D-galactosamine/lipopolysaccharide (D-GalN/LPS) model of acute hepatitis. Study findings indicated lower serum alanine aminotransferase (ALT) levels, increased serum and tissue TNF-α levels, and decreased histologic findings of liver damage in the Fat1 mice as compared to wild-type litter mates 6 hours after D-GalN/LPS injection.

Essential fatty acids (EFA) are termed as such because they cannot be synthesized by the human body and thus must be derived from exogenous sources. There are 2 EFA groups—omega-6 and omega-3. The biologically active omega-6 fatty acid is arachidonic acid (AA). The downstream omega-3 products are eicosapentaenoic acid (EPA) and docosahexaenoic acid. All of these fatty acids play an important role in cell membrane composition, thus influencing fluidity and cell surface biochemical signaling, as well as serving as natural ligands for certain nuclear receptors that affect gene expression. In addition, AA and EPA are important eicosanoid and prostanoid precursors. Arachidonic acid products are proinflammatory mediators, whereas EPA products are antiinflammatory or rather less proinflammatory [19-22]. This makes their interaction critical, especially considering that AA and EPA are competitive substrates.

Our hypothesis is that omega-3 fatty acid supplementation reduces hepatocellular damage and cell death in a model of murine common bile duct ligation, an animal model for cholestatic diseases such as biliary atresia, and to characterize the mechanism by which this may occur.

1. Materials and methods

1.1. Murine common bile duct ligation

Twenty disease-free 6 to 8-week-old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, Me) were divided into 2 groups and fed either 5% soybean oil or 10% Menhaden oil in a purified rodent diet (Dyets Incorporated, Bethlehem, Pa) (Table 1) for 3 weeks before surgery. The animals, in groups of 5, were housed in a barrier room and were acclimated to their environment for at least 72 hours before the initiation of each experiment. Common bile duct ligation was performed under 3% isoflurane anesthesia. A midline laparotomy, extending from xyphoid to pubis, was made. The duodenum

Table 1 Fatty acid content of Menhaden and soybean oil

Fat composition (%)	Menhaden oil	Soybean oil
Linoleic	1.5	54.8
α-Linolenic	1.6	7.8
Arachidonic acid	0.9	-
EPA	15.5	-
DHA	9.1	-
Oleic	11.4	22.7
Palmitic	17.1	10.2
Stearic	2.8	4.5

DHA indicates docosahexaenoic acid.