A New Perspective on Fish Oil: The Prevention of Alcoholic Liver Disease

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Abstract: The mechanisms of alcoholic liver diseases (ALD) are very complex and interrelated, including abnormal lipid metabolism, oxidative stress, and gut-derived endotoxin pathway. On the other hand, fish oil is rich in n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which decrease blood triglyceride concentration in hypertriglyceremia patients and show protective effects against fatty liver. However, there is limited evidence from studies of the relationship between fish oil and ALD based on the viewpoint of the intestinal integrity and microflora. Therefore, this review discusses the mechanism of amelioration for ALD by fish oil. Based on our previous studies, partial replacement of olive oil by fish oil in alcohol-containing liquid diet ameliorated the liver damage including fatty liver and inflammation in rats. Based on these results, the mechanisms of hepatoprotective effects due to fish oil substitution were discussed in three parts, such as regulating lipid metabolism, decreasing oxidative stress and maintaining intestinal health. First of all, we found that fish oil substitution increased plasma adiponectin levels, and then increasing MCAD and CPT-1 mRNA levels to accelerate fatty acid oxidation in liver, then further prevent ethanol-induced hepatosteatosis in rats with chronic alcohol-feeding. Fish oil replacement also enhanced hepatic autophagy flux, which enhanced lipid degradation, then inhibited lipid accumulation in liver. Secondly, the appreciable proportion of fish oil decreased lipid peroxidation by reducing the protein expression of cytochrome p450 2E1 in chronic alcohol-feeding rats. We also speculated that the appropriate proportion of n-6 and n-3 PUFAs is very important for preventing alcoholic liver disease. At last, substituting fish oil for olive oil normalized the intestinal permeability and fecal microbiota composition, thus providing a low plasma endotoxin level and inflammatory responses, which exert ameliorative effects on ethanol-induced liver injuries in rats.

Key words: alcoholic liver disease, fish oil, lipid metabolism, oxidative stress, inflammation, gut permeability, microbiota composition

1 Introduction

Alcohol abuse is a health-related burden that occurs worldwide. A report announced by World Health Organization (WHO) in 2018, indicated that the current drinking population is around 45%. As shown by the WHO data, total alcohol per capita consumption among drinkers is 15.1 L of pure alcohol per year. Namely, every adult drinker consumes almost 32.8 g of pure ethanol each day. Social drinking or moderate alcohol consumption for many situations is pleasurable. However, excess alcohol consumption is linked to a number of negative outcomes, such as a risk factor for diseases and health impacts, crime, road incidents and alcohol dependence. The global alcohol consumption causes 2.8 million premature deaths per year in the world.

In Taiwan, the drinking population who is more than 18 years old is around 45%2. Most of drinkers is distributed from 18 to 49 years old2. The alcohol consumption was
also increased from 2009 to 2017 and maintained in the high level consumption in recent years in Taiwan\(^2\). Moreover, the top three of ten leading causes of cancer death are lung, liver and colon cancers in Taiwan in 2019\(^3\). It maintains same ranking more than 10 years. Liver is a "silent organ", because it can be damaged without sending any signals or symptoms. Some of symptoms of liver disease may be non-specific to the liver and often patients have few or no signs of any liver disease at all, which makes liver diseases more dangerous. On the other hand, Lo et al. indicated that the risk factors of chronic liver disease were hepatitis B virus, alcohol use and smoking in 2009 in Taiwan\(^5\). Moreover, some research indicated that the patients with virus hepatitis who drank a lot of alcohol may lead to develop cirrhosis, because excessive alcohol promoted the reproduction of virus and made the hepatic cirrhosis more serious\(^5\). Taiwanese people lack acetaldehyde dehydrogenase isoenzyme 2 (ALDH2), which is the enzyme to metabolize acetaldehyde to acetic acid\(^7\). As a result, the prevention of alcoholic liver disease (ALD) is an important issue in Taiwan, because of the high infection of hepatitis virus, the increased consumption of alcohol and the mutation of ALDH2.

Fish oil contains abundant amounts of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which were found to play important roles in lipid metabolism, antioxidative status, and immune function\(^10\). However, the effects of fish oil on ALD are still controversial. The possible reason is that fish oil was used as the only dietary source in many animal studies\(^8\)–\(^11\). In our previous studies, we found that hepatic steatosis and inflammation were ameliorated when fish oil was added to replace partial olive oil in rats fed with an ethanol-containing liquid diet for 8 weeks\(^12\)–\(^16\). Therefore, this article reviewed that preventive effects and possible mechanisms of fish oil on alcoholic liver diseases based on the lipid metabolism, oxidative stress and gut health, including permeability and microbiota composition.

## 2 Pathogenesis of ALD

### 2.1 The spectrum of ALD

Alcohol consumption leads to hepatocellular damage via ethanol metabolic mechanisms and may progress to ALDs, including fatty liver, hepatitis, cirrhosis, and even hepatocellular carcinoma\(^7\). Around 90% of heavy drinkers has steatosis\(^7\)–\(^9\). This stage is reversible when alcohol use stops. Risk factors, such as gender, drinking pattern, obesity, viral hepatitis, and genetics can contribute to ALD progression\(^7\)–\(^9\),\(^17\)–\(^20\). About 20%-40% of patients with steatosis will progress to alcoholic steatohepatitis\(^8\)–\(^9\). Some of those patients will develop liver fibrosis, cirrhosis and hepatocellular carcinoma\(^7\)–\(^9\),\(^17\)–\(^20\).

### 2.2 The pathogenic mechanism of ALD

The pathological mechanisms of ALD are complicated. Many factors are involved in ALD and interact with each other. First of all, chronic alcohol intake disturbs the lipid metabolism and cause lipid accumulation in liver\(^20\). Secondly, chronic alcohol intake also induces drug metabolized system, such as cytochrome P450 2E1 (CPY2E1), then causes oxidative stress\(^20\)–\(^21\). Recently, many papers also reported that chronic alcohol intake increased the permeability of the gut mucosa and changed the gut microbiota composition, which caused high blood endotoxin level and inflammation\(^20\),\(^22\).

### 2.3 The treatment of ALD

Abstinence from alcohol and lifestyle modification are the paramount step for the management of ALD. Reducing alcohol consumption leads to resolution of alcoholic fatty liver and improvement of survival in alcoholic cirrhotic patients\(^23\),\(^24\). Pharmacotherapy is also used to diminish symptoms depends on the different stages of liver diseases. For example, steroids was to inhibit the pro-inflammatory transcription factors such as activator protein 1 (AP-1) and nuclear factor-kB (NF-kB), which leads to benefit in patients with severe alcoholic hepatitis\(^25\). Pentoxifylline and specific anti-TNF-α therapy are used to improve outcomes in alcoholic hepatitis via downregulation of pro-inflammatory cytokines that are thought to play a role in the pathogenesis of alcoholic steatohepatitis (ASH) and are known to be elevated and correlate with disease severity\(^26\),\(^27\). Pentoxifylline has also been shown to have antifibrotic effects through the attenuation of both profibrogenic cytokine and procollagen I expression\(^28\).

## 3 Nutritional Support for ALD

### 3.1 Malnutrition in ALD

It has been established that most of patients with ALD is under malnutrition which is related with the severity of liver damage\(^29\). Moreover, the complications of ALD, such as infections, encephalopathy, ascites and variceal bleeding, may be caused by protein-calorie malnutrition\(^30\). Deficiencies of vitamins (folate, thiamine, vitamin B6, vitamin A) or minerals (selenium, zinc, copper, and magnesium) are also commonly happened in ALD\(^30\).

### 3.2 Nutrition-related alternative medicine

The nutrition-related alternative medicine links to ALD can be classified by the pathological mechanism. Several studies already indicated that some saturated fatty acid, such as MCT oil, cocoa butter or lard inhibited the lipid accumulation in liver in rats or mice fed with alcohol, although the results are still controversial\(^3\)–\(^11\). Moreover, antioxidants can protect against the oxidative stress induced
by alcohol, accompanied with anti-inflammation, anti-apoptosis, and autophagy regulation effects, such as milk thistle\textsuperscript{31}, betaine\textsuperscript{30}, β-carotene\textsuperscript{31, 30}. In addition, some nutrients can prevent ALD by improving the intestinal permeability and microbiota composition in rats fed with alcohol, such as glutamine\textsuperscript{35}, epidermal growth factor (EGF)\textsuperscript{36}, probiotics\textsuperscript{37} or synbiotics\textsuperscript{38}. Although a multitude of animal studies have been demonstrated, clinical trials in human subjects are necessary.

4 Fish oil and ALD

4.1 Fish oil and alcohol-induced liver damage

The effects of fish oil on the ALD are inconsistent based on the previous studies. Poly unsaturated fatty acids (PUFAs), such as corn oil or fish oil, have been shown to exacerbate experimental models of alcoholic liver injury by increasing oxidative stress, whereas saturated fatty acids are protective\textsuperscript{39, 40}. It was speculated that fish oil used as the only one dietary fat in the previous studies which was associated with oxidative stress and essential fatty acid deficiency. Moreover, this dietary composition used in the animal studies cannot be applied to human daily diet, because it is impossible to ingest fish oil as the only one dietary fat sources. Therefore, the modification of dietary fatty acid composition by fish oil is suggested for preventing ALD. In our studies, the dietary fatty acid composition was adjusted with fish oil substituted for 25% or 57% olive oil in liquid diet which contained alcohol (36% of total energy) and fat (olive, corn oil and safflower oil, 36% of total energy)\textsuperscript{12-16}. One gram fish oil contained 350 mg EPA and 250 mg DHA. The ratios of n-6 to n-3 were 2.2 and 1.0, respectively\textsuperscript{41}. Rats were fed with the alcohol-containing liquid diets with or without fish oil substitution for 8 weeks. It was found that hepatic lipid accumulation, inflammation and lipid peroxidation were decreased by fish oil replacement in rats with chronic ethanol-feeding\textsuperscript{12-16}. In an acute toxicity of ethanol administration model, fish oil could reduce ethanol-induced fatty liver and hepatic production of the inflammatory cytokines, because EPA can be converted into anti-inflammatory prostaglandins (PGs) of the PGE3 series\textsuperscript{17}.

4.2 Lipid metabolism and biological regulators

Adiponectin is secreted from adipose tissues, then combines with adiponectin receptor 2 (adipoR2) on hepatic membranes to activate AMP-activated protein kinase α (AMPKα) and NAD-dependent deacetylase sirtuin-1 (SIRT1) which regulates the factors of lipidogenesis and lipolysis in the downstream\textsuperscript{42, 41}. It was indicated that plasma adiponectin levels and hepatic adipor2 expression were reduced after chronic ethanol intake, which might inhibit the activation of hepatic AMPKα and SIRT1\textsuperscript{43}. The inactivation of hepatic AMPKα and SIRT1 caused the elevation of mRNA expressions of sterol response element-binding protein (SREBP)-1c, which is thought as a transcription factor of lipogenesis. SREBP-1c elevates the enzyme activities which are related with fatty acid synthesis, including fatty acid synthase (FAS), acetyl co(enzyme)A carboxylase 1 (ACCoA) and stearoyl coenzyme A desaturase (SCD)-1, then to enhance hepatic lipid synthesis\textsuperscript{44}. Moreover, inactivation of hepatic AMPKα and SIRT1 also decreased peroxisome proliferator-activated receptor (PPAR)-α transcriptional activity and decreased medium-chain acyl-CoA dehydrogenase (MCAD) and acyl-CoA oxidase 1 (ACO1) mRNA levels that are associated with lipolysis\textsuperscript{45}. In addition, inactivated AMPK α can decrease ACC1 phosphorylation, and then increase ACC1 activity to produce malonyl-CoA. The elevation of malonyl-CoA might decrease CPT-1 activity to suppress hepatic fatty acid oxidation\textsuperscript{46}. However, we indicated that fish oil substitution for olive oil could inhibit the reduction of plasma adiponectin and elevate hepatic CPT-1 and MCAD mRNA levels, which improved fatty acid oxidation and further prevented ethanol-induced hepatic steatosis in rats\textsuperscript{44}. Meanwhile, it was also found that there was a positive correlation between the ratio of n-6 to n-3 and the hepatic inflammation, and negative correlations between the ratio of n-6 and n-3, and plasma adiponectin in rats fed with ethanol\textsuperscript{47}.

4.3 Autophagic flux

Many studies also reported that autophagy was related with cellular-degradation procedure such as lipid droplets or damaged mitochondria\textsuperscript{48}. Poor autophagy will cause fat accumulation in liver. There are four main steps involved in the process of autophagic flux, including induction, autophagosome formation, lysosome fusion and breakdown. The inhibition of mTOR (mammalian target of rapamycin) induced autophagy, then, the complex coordinates actions of ATGs, especially ATG6 (Beclin 1) and ATG8 (light chain 3; LC3), engulfs the double membrane-bound autophagosome\textsuperscript{47-49}. Autophagosomes move to fuse lysosomes by microtubule activation to form an autolysosome which are degraded by lysosomal enzymes and serve as a catabolic energy source\textsuperscript{47-49}. During degradation of the autolysosome, the internal membrane protein, sequestome-1 (p62) is also degraded\textsuperscript{47}. Therefore, Beclin-1, LC3II and P62, LC3 and p62 are considered markers for monitoring autophagic flux, which are involved in the autophagosome formation and breakdown\textsuperscript{47-49}. It was indicated that chronic alcohol consumption suppresses autophagy, which is related to liver damage; however, the mechanisms responsible for retarding autophagy are not clear\textsuperscript{47}. On the other hand, a recent study reported that fish oil can increase hepatic LC3II/LC3I and Beclin1 protein expressions and decrease p62 expression when activating autophagic flux in Wistar rats\textsuperscript{50}. In our previous study, it was also found that using...
fish oil to substitute for olive oil in the ethanol-containing liquid_diet significantly improved hepatic autophagic flux, such as up-regulation of LC3II, Beclin1, and down-regulation of p62 protein expression which was closely associated with the hepatic protection by fish oil in rats with chronic ethanol-consumption13.

### 4.4 Gut health

Recent studies suggested that alcohol consumption destroyed the intestinal membrane and disrupted the gut microbiota composition which allowed gram negative bacteria-derived products, such as lipopolysaccharides (LPSs), to pass into the systemic circulation thereby inducing endotoxemia. Through the gut-liver axis, LPS entered into the liver through portal and combined with toll-like receptor 4 (TLR4), then activated the immune system via MYD88 and TRIF signaling pathway to induce alcoholic hepatitis, as confirmed in experimental ALD animal models54, 55.

Previous study found that n-3 PUFAs changed the lipid environment in tight junction membrane microdomains, prevented the redistribution of tight junction proteins20. However, some studies failed to find the beneficial effects of fish oil on the protein expression of intestinal tight junction protein, ZO-1 in rats with alcoholic liver injury15. A previous study also demonstrated that the signaling and transport processes for endotoxin are initiated in specialized membrane microdomains called lipid rafts, and oil rich in n-3 PUFAs may unsettle lipid rafts that inhibit greater endotoxin transport54, 55. Therefore, further studies are necessary to clarify the improvement effects of fish oil on intestinal structural integrity which is closely linked to permeability.

**Firmicutes** and **Bacteroidetes** abundances represent 60% of the intestinal microbial community, which can largely impact imbalances in gut microbiotic communities56, 57. Currently, it could not be found a clear trend between the F/B ratio and ALD. Past studies noted that there was an increase in the Firmicutes and a reduction in the Bacteroidetes in ALD cirrhosis patients90. However, other studies showed reduced Firmicutes abundance in alcohol-fed mice via intragastric administration90 and by a feeding tube90. Therefore, it was speculated that the different results were caused by the ethanol-treatment routes. In our study, it was found that the F/B ratio showed the significantly higher in rats fed with ethanol-containing liquid diet, which was similar with the results in patients ALD cirrhosis12. Therefore, the liver damage induced by an ethanol-containing liquid diet more closely mimicked ALD in humans.

In view of the beneficial actions of the gut microbiota and PUFAs on various diseases, such as diabetes mellitus, inflammatory bowel disease, coronary heart disease, and cancer, it was speculated that the beneficial effects of fish oil were due to the regulation of gut microbiota composition61, 62. Liu et al. indicated that high n-3 PUFAs diet boosted the proportion of species of the *Bacteroidetes* phylum30. Our previous study also showed that fish oil replacement could inhibit elevation of the F/B ratio induced by ethanol feeding in rats12.

At the family level, several bacterial communities were significantly increased in ALD rodent model and patients, including *Porphyromonadaceae*, *Rikenellaceae* and *Prevotellaceae*12, 64. *Porphyromonadaceae* and *Prevotellaceae* have been indicated to be correlated with inflammation64. At the generic level, a high level of *Alistipes* were found not only in rats with alcohol-induced liver damage but also in mice receiving fecal microbiota from alcoholic patients with severe hepatitis12, 65. It was also demonstrated that *Alistipes* was a potential acetaldehyde accumulator in the gut65.

There is few study to clarify the relationship between gut microbiota and PUFAs. Several studies indicated that fish oil increased the *Actobacillaceae* which is thought as the producer of short-chain fatty acids (SCFAs)12, 66. It has been indicated that SCFAs played important roles in the metabolic regulation which was linked to reduce the risk of gastrointestinal disorders, cancer and cardiovascular disease67. Using the culture method for the analysis of specific bacteria species, *E. coli*. as the indicator of harmful bacteria, was elevated in rats with chronic alcohol-feeding, but decreased in rats when replaced a partial of olive oil with fish oil67. Furthermore, the beneficial bacteria, *Bifidobacterium* was decreased rats fed with alcohol and increased in rats fed with fish oil containing-alcohol diet16. The effects of fish oil on the gut microbiota composition in rats with alcohol-induced liver damage must be discussed in the future study.

### 4.5 Dosage information of ethanol and fish oil

The daily ethanol intake of ethanol was around 9-9.5 g/kg BW /rats based on the research which used ethanol-containing liquid diet to induce ALD12-16. According to the “Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers” announced by the Center for Drug Evaluation and Research (CDER), the conversion of animal doses to human-equivalent doses based on the body surface area is 0.16. Therefore, the daily ethanol intake level of rats is the equivalent to 86.4 ~ 91.2 g/day in a human weighing 60 kg (9 × 0.16 × 60 = 86.4; 9.5 × 0.16 × 60 = 91.2) which is considered to be a heavy alcohol intake (more than 50 ~ 60 g/day)68.

As mentioned above, the hepatoprotective effects of fish oil in ethanol-induced animal models were inconsistence because fish oil was commonly used as the only one dietary fat source in the previous studies9-11. In contrast, when fish oil was used as the partial dietary fat source, alcohol-induced liver damage was observed in our studies12-16. However, fish oil substitution did not demonstrate a dose-
response relationship in ameliorating alcohol-induced liver damage according to the direct evidence of liver damage from the histopathological analysis. Our previous study also indicated that the range of n-6/n-3 fatty acid ratios which was positively correlated with inflammatory cell infiltration in rats subjected to chronic ethanol feeding. Therefore, it could be speculated that the hepatoprotective effects of dietary fish oil might be strongly related to the reduction in the hepatic ratio of n-6/n-3 fatty acids in the liver. Moreover, the recommended dosage of n-6/n-3 fatty acid ratios was 1.0 to 2.2 by fish oil replacement based on our past studies.

5 Summary

The effects of PUFAs on ALD are still controversial. We have finished a series of study to clarify the protective effects of fish oil on ALD via the different pathogenic mechanisms. When fish oil was used to adjust the dietary fatty acid composition and the ratio of n-6 and n-3 in rat with alcohol feeding, it was found that the lipid metabolism was improved, the oxidative stress was decreased and the inflammatory reaction was inhibited (Fig. 1). That is to say, fish oil has the potential in preventing ALD. Furthermore, the preventive effects of fish oil may be due to the promotion of lipolysis and autophagy, elevation of anti-oxidative ability, and improvement of gut permeability (Fig. 1). Based on the previous studies, it was suggested that the dietary fat sources should be considered for heavy drinkers, especially the ratio of n-6 to n-3 PUFAs for preventing the hepatic injury.

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