Fucoidan in the Prevention and Treatment of Chronic Disease

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Introduction

Marine algae have long been a part of food and diet. Algae have also been documented as being used in oriental medicine for more than 1,000 years (Smit, 2004; Wijesekara, 2010). All classes of algae contain polysaccharides in their cell walls, which can be commonly found in food and cosmetics, as compounds such as carrageenans, alginates and agar. Marine algae are classified according to the type of pigment they contain. These include Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae) (Barros Gomes Camara, et al., 2011). The class Phaeophyta, in particular, contains sulfated polysaccharides, termed fucan or fucoidan (heterofucans), which are becoming increasingly studied in biomedical research. Each fucoidan is structurally unique, so the relationship between structure and biological activity has not yet been found (Barros Gomes Camara, et al., 2011).

According to Tutor Ale, Mikkelsen and Meyer, Fucans were first isolated from algae in 1913, and since then, approximately 1,800 studies have been published on the topic, with a significant increase in the last five to ten years (2011). This increase in fucoidan interest can be credited to the polysaccharide’s potential health benefits, such as antioxidant, anti-inflammatory, anticoagulant, antithrombotic, antitumor, and antiviral (Tutor Ale, et al., 2011). Recently, there has been a rise in related chronic conditions, such as cardiovascular disease (CVD), diabetes and cancer, in part due to lifestyle factors, aging and urbanization (Barros Gomes Camara, et al., 2011). The current treatments for these diseases are often painful and have adverse side effects, so the need for alternative therapies can also be attributed to the increase in fucoidan research.

Despite this plethora of research and medical interest, the mechanism behind fucoidan’s beneficial activities has yet to be fully understood and applications in
treatment of diseases have not yet been approved for fucoidan. Extraction and purification methods vary across studies, and the chemical structures of the extracts vary, as well (Tutor Ale, et al., 2011). For this reason, it is important to note the species of algae and extraction methods used in fucoidan research.

**Anticoagulant and Antioxidant Activities**

According to the Centers for Disease Control and Prevention (CDC), CVD is the leading cause of death in the United States, with about 600,000 adults dying from the disease, each year (2013). Related conditions, cancinogenesis and diabetes, also make the CDC’s list of leading causes of death, with 574,743 yearly deaths from cancer and 69,071 from diabetes (2013). Although the causes these diseases cannot be narrowed down to one single factor, Freinbichler, et al. claim that oxidative stress and the resulting inflammation has been linked to diabetes, atherosclerosis and cancinogenesis (as cited in Barros Gomes Camara, et al., 2011). Oxidative stress can be prevented through the use of antioxidants. Several synthetic antioxidants have been developed and are commonly used in food products. These include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ). Such antioxidants have shown to be effective in preventing lipid peroxidation, however, according to Hu, et al., the synthetic compounds have possible cancinogenic and toxic health effects (as cited in Barros Gomes Camara, et al., 2011). Furthermore, fucan has showed promise as an antioxidant in several applications and has no adverse health effects (Barros Gomes Camara, et al., 2011).

In atherosclerosis and CVD, anticoagulants are used to prevent thromboembolic disorders. Some of the most commonly used anticoagulants are unfractionated heparins and their low weight derivatives (Barros Gomes Camara, et al., 2011). Unfractionated
Heparins are sulfured polysaccharides, which inactivate the clotting factor, thrombin (Hirsh, et al., 2001). According to Barros Gomes Camara, et al., the drug has several negative side effects including “frequent activated partial thromboplastin time monitoring, variable anticoagulant effects, the inability to inhibit clot-bound thrombin and the occurrence of thrombocytopenia, all of which have led to the search for alternative sources of anticoagulant agents” (2011).

In 2011, Barros Gomes Camara, et al. published a study in Brazil, which sought to isolate sulfated polysaccharides from Canistrocarpus cervicornis, and evaluate their anticoagulant and antioxidant activities, in vitro. The C. cervicornis was collected from Búzios Beach, Nísia Floresta, Brazil. The samples were then dried in an oven, ground in a blender, and incubated with acetone to remove lipids and pigments. Next the samples underwent a pH adjustment, proteolytic enzyme digestion and further incubation. It was then filtered through a cheese cloth and fractionated, so the resulting precipitate could be collected by centrifugation, vacuum dried, re-suspended in distilled water, and stained with toluidine blue, to reveal that the samples consisted solely of sulfated polysaccharides (Barros Gomes Camara, et al., 2011).

Six families of heterofucans were obtained from C. cervicornis, denoted CC-0.3, CC-0.5, CC-0.7, CC-1.0, CC-1.2, and CC-2.0 (see Appendix A, Figure A1) (Barros Gomes Camara, et al., 2011). Anticoagulant activity was measured using prothrombin time (PT) and activated partial thromboplastin time (aPPT) coagulation assays. In the PT test, no clotting activity was found for any of the families. In contrast, each family extended aPPT, in a dose-dependent manner. “Heterofucans CC-0.3, CC-0.5, CC-0.7, and CC-1.0 doubled aPPT with only 0.1 mg/mL of plasma, a result similar to that of Clexane®, a commercial low molecular weight heparin that had the same effect with 0.08
mg/mL of plasma” (Barros Gomes Camara, et al., 2011). Researchers also concluded that heterofucans act via the intrinsic pathway for coagulation and effects are specific to the sulfation site and/or glycosidic linkage within fucoidan, not sulfate charge density or amount (Barros Gomes Camara, et al., 2011).

Antioxidant activity was evaluated using total antioxidant capacity, superoxide radical scavenging, hydroxyl radical scavenging, and metal-chelating property. Although none of the heterofucans showed significant antioxidant effects on hydroxyl radicals, it is important to note that CC-1.2 showed excellent superoxide radical scavenging activity (see Figure A2) (Barros Gomes Camara, et al., 2011). The superoxide radical is a precursor to the hydroxyl radical; therefore, heterofucans may be able to inhibit this radical by preventing its formation. For the metal-chelating property test, antioxidant activity was varied among the heterofucans, with CC-0.5, CC-0.7, and CC-1.0 showing the best capacity (see Figure A3) (Barros Gomes Camara, et al., 2011). The researchers concluded that heterofucans, specifically CC-0.7 and CC-1.2, have strong anticoagulant and antioxidant activities, and therefore are “potential multipotent drugs” (Barros Gomes Camara, et al., 2011).

**Antitumor and Antimetastatic Activities**

Not only does fucoidan show promise as an antioxidant, which can help prevent cancer, but it also has shown the potential to prevent the spread of existing cancer cells. Metastasis occurs when cancer cells break off the primary tumor and move through the blood and lymphatic vessels to invade other organs. This phenomenon causes up to 90 percent of cancer-related deaths (Lee et al., 2012). Matrix metalloproteinases (MMPs) play a key role in the mechanism behind tumor metastasis, by degrading extracellular matrix proteins such as collagen, proteoglycan, elastin, laminin, and fibronectin. Cancers
with the highest levels of MMPs, such as lung cancer, are particularly aggressive and metastatic (Lee et al., 2012).

Due to the harsh nature of current antitumor treatments, such as chemotherapy, more research is being conducted to identify potential therapies from natural sources (Lee et al., 2012). A South Korean study conducted by Lee, J. Kim, and E. Kim (2012) sought to assess and understand the mechanism of fucoidan anti-metastatic effects, using a highly metastatic form of human lung cancer, denoted A549. For this study, fucoidan extract was obtained from *Fucus vesiculosus*, of Sigma, St. Louis, Missouri. The powder was dissolved in phosphate buffer solution and sterilized, using a filter. First, Twelve-well plates were inoculated with A549 cells, and allowed to grow for 24 hours. After incubation, non-invading cells were removed, and the remaining invasive cells were inoculated with various concentrations of fucoidan and incubated for 48 hours (Lee et al., 2012).

At concentrations of 400-1,000 µg/ml, “fucoidan inhibited cell proliferation and decreased cell viability in a concentration-dependent manner” (see Figure B1) (Lee et al., 2012). At a concentration of 200 µg/ml, fucoidan reduced MMP-2 activity by 72 percent and inhibited MMP-2 protein expression by 60 percent (see Figure B2) (Lee et al., 2012). The effects of fucoidan on A549 cell invasion were evaluated using an invasion chamber kit. At 200 µg/ml, cell invasion was inhibited by 86 percent (see Figure B3) (Lee et al., 2012). Finally researchers found that fucoidan inhibited several pathways by which cancer cells migrate and invade in humans, such as the ERK1/2 and PI3K pathways. From these results the researchers concluded that because fucoidan is indeed an anti-metastatic reagent and shows low side effect in normal cells, further in vivo studies and clinical investigations are needed to apply fucoidan as a new cancer therapy.
Antiviral Activities

Fucoidan’s inhibitory abilities extend beyond just tumor inhibitions. A study by Baba, Snoeck, Pauwels, and de Clercq (1988) found that fucoidan has the potential to inhibit viral replication including herpes simplex virus and human immunodeficiency virus (as cited in Mori, Nakasone, Tomimori, & Ishikawa, 2012). Hepatitis C virus (HCV) currently has infected about 170 million people globally, with an estimated 3.5 million people newly diagnosed, each year (Mori, et al., 2012). This virus is extremely virulent, devastating to the liver, and chronic infection can cause liver cirrhosis (LC), which can lead to liver cancer (Chen, et al., 2013). HCV strains vary greatly, so no vaccine currently exists. Current antiviral treatments can be sometimes results in serious side effects and a significant portion of those treated are non-responders (Mori, et al., 2012).

In Japan, Mori, et al. conducted a study, which assessed the antiviral activity of fucoidan on HCV, both in vivo and in vitro (2012). The fucoidan used was extracted from Cladosiphon okamuranus Tokida, cultivated from Okinawa, Japan. The alga was cooked in a water-acid solution, neutralized and cooled, centrifuged, filtered, then dried. Study participants consisted of 15 chronic liver disease patients, all infected with HCV. Six of the patients were non-responders to standard treatments and six suffered from LC (Mori, et al., 2012).

Researchers found from the in vitro testing that fucoidan inhibited HCV replication, as indicated by luciferase (LUC) activity, but did not exhibit cytotoxic effects, as cell viability was not significantly affected (see Figure C1) (Mori, et al., 2012). These results were similar to those found in vivo. There was no significant decrease in HCV RNA levels or clinical symptoms in the participants, however, none of the patients
showed progression of LC during the 12-month treatment (see figure C2). It is important to note, however, that the patient pool was small and contained non-responders to standard treatments (Mori, et al., 2012).

In Taiwan, a similar study was conducted by Chen, et al., which assessed the effects of *Gracilaria tenuistipitata* (AEGT) extract on HCV replication, in vitro, and indentified the mechanism behind these effects (2013). The extracts were cultivated from Kouhu beach, Taiwan, soaked in distilled water and dried. They were then pulverized and filtered. The researchers found that AEGT inhibited HCV protein synthesis and RNA synthesis in a dose-dependent manner however; these effects were not cytotoxic (see Figure C3). The researchers then combined AEGT with the standard HCV treatment, pegylated-interferon-α (IFN-α), which resulted in synergistic inhibition. Researchers concluded that AEGT was useful in treatment and prevention of chronic HCV infection, however, since a crude extract was used, further purification would be necessary for future studies (Chen, et al., 2013).

**Extraction Methods and Molecular Structure**

Although fucoidan has shown immense potential for the treatment of aforementioned chronic diseases, the relationship between the beneficial activities of fucoidan and the structure of the molecules has not yet been completely understood. According to a review by Tutor Ale, et al., this understanding has been delayed by the lack of extraction and purification standards and protocol in fucoidan research (2011). The authors also state that the methods of extraction affect the final structure of the fucoidan molecules, which has an effect on their biological properties. It has been noted across various studies, that the degree of sulfation and size of the fucoidan molecules has a direct impact on their antitumor and anticoagulant properties (Tutor Ale, et al., 2011).
Tutor Ale, et al., suggest that standard extraction procedures should be developed, which include “hydrolysis treatment, purification and fractionation methodology, preferably with specific steps adapted to the particular botanical order of the seaweed” (2011).

**Conclusion**

Current research has shown that fucoidan, extracted from brown algae, has anticoagulant, antioxidant, antitumor, and antiviral properties. Fucoidan is a potential effective treatment for chronic disease, such as CVD and cancer, and is a more healthful alternative to current treatments. Despite ample research on the topic, standard protocol for fucoidan extraction and purification must be developed, so that fucoidan can be better understood and eventually approved for use in disease prevention and treatment (Tutor Ale, et al., 2011).
References


Appendix A

Fucoidan Anticoagulant and Antioxidant Activities

Figure A1

“Anticoagulant activity by aPTT test. Results were expressed as aPTT ratio, obtained by dividing clotting time achieved with the anticoagulant by that obtained with the control. Data are expressed as means ± standard deviation of four determinations; a,b,c,d Different letters indicate a significant difference between each concentration of the same sulfated polysaccharide using one-way Anova followed by the Student-Newman-Keuls test (p < 0.05). Cx: Clexane®” (Barros Gomes Camara, et al., 2011)

![Graph](image)

Figure A2

“Total antioxidant capacity of sulfated polysaccharides extracted from the marine brown seaweed C. cervicornis. The results are expressed as ascorbic acid equivalents. Each value is the mean ± SD of three determinations: a,b,c Different letters indicate a significant difference (p < 0.05) between sulfated polysaccharides” (Barros Gomes Camara, et al., 2011).
Figure A3

“Ferrous chelating activity of sulfated polysaccharides from the brown seaweed C. cervicornis. Each value is the mean ± standard deviation of three determinations: a, b, c. Different letters indicate a significant difference (p < 0.05) between each concentration of the same sulfated polysaccharide” (Barros Gomes Camara, et al., 2011).
Appendix B

Fucoidan Antimetastatic and Antitumor Activities

“The data shown are the means ± SD of three experiments. Significant difference from control group, *p<0.05 and **p<0.01” (Lee et al., 2012).

Figure B1

“MMP-2 activity by analysis of zymography was quantified by measuring the band intensities using Image J software” (Lee et al., 2012).
Figure B2

“Expression of MMP-2 by analysis of western blot was quantified by measuring the band intensities using Image J software” (Lee et al., 2012).

Figure B3

“The amount of invading cells was quantified using Image J software” (Lee et al., 2012).
Appendix C

Fucoidan Antiviral Activity

Figure C1

“Anti-hepatitis C virus effects of fucoidan in hepatitis C virus replicon cells. Luciferase (LUC) activity (a marker of replication level) and cell viability of FLR3-1 cells, which constitutively express hepatitis C virus replicon, were measured in the presence of various concentrations of fucoidan. LUC and WST-8 assays were performed in triplicate. Data are mean ± SD” (Mori, et al., 2012).

Figure C2

“Effects of treatment with fucoidan on hepatitis C virus RNA and alanine aminotransferase levels in patients with liver diseases. Serum alanine aminotransferase (ALT) levels, indicative of HCV RNA activity. Values are mean ± SD. bP < 0.01 vs pretreatment value” (Mori, et al., 2012).
“Concentration-dependent reduction of HCV RNA replication in AEGT-treated HCV replicon cells. HCV RNA levels were quantified by qRT-PCR and normalized to cellular gapdh mRNA levels following AEGT treatment for 3 days. Cellular toxicity was evaluated by the MTS assay after 3 days of incubation with AEGT” (Chen, et al., 2013).