University of Nevada, Reno

Decreasing Omega-6 to Omega-3 Polyunsaturated Fatty Acid Ratios Inhibit Tumorigenesis in Prostate Cancer Cells in Vitro

A thesis submitted in partial fulfillment

of the requirements for the degree of

Bachelor of Science in Biochemistry and the Honors Program

by

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We recommend that the thesis
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Abstract:

Prostate cancer is the most commonly diagnosed cancer in American men. Currently, there are different treatment options depending on the situation, for instance, hormone therapy is a common treatment which reduces the levels of androgens. However, prostate cancer that is more aggressive or in an advanced stage can become unresponsive to hormonal treatment. This type of prostate cancer is referred to as hormone refractory prostate cancer. In many epidemiology studies, they have shown that diets low in omega-6 polyunsaturated fatty acids (PUFAs), and rich in omega-3 PUFAs display a lower incidence of cancer. A particular type of omega-6 fatty acid, docosahexaenoic acid (DHA), has shown to decrease the growth of cancer cells. Therefore, DHA may have potential therapeutic effects on treating cancer. However, the current diet consists of low omega-3 content while very high omega-6 content. For example, the western diet (United States) consists of a ratio of about 16:1 (omega-6 to omega-3). In this tissue culture study, varying ratios of omega-6 to omega-3 polyunsaturated fatty acids (PUFAs) were tested to determine which ratio representing a human diet inhibited growth of PC-3 human prostate adenocarcinoma cell the most, which is a hormone refractory prostate cancer. The results showed that as the ratio decreased, the growth of prostate cancer cell decreased. The most optimal ratio was found to be 1:2 omega-6 to omega-3. However, the 4:1 ratio displayed the most practical dietary ratio to inhibit prostate cancer.

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Introduction:

The significance of nutrition on cancer was first noted when epidemiological studies showed diets rich in omega-3 polyunsaturated fatty acids (PUFAs) resulted in lower incidences of cancer (3, 5, 13). Since then, much research has been done investigating the effects of omega-3 PUFAs on cancer cell growth. For instance, omega-3s have anti-inflammatory effect, anti-proliferative effect, and anti-angiogenic effect, leading to inhibiting cancer growth (15). Though it has been widely confirmed that omega-3s show anti-cancerous effects and tumor-suppressing activity compared to omega-6s, a common feature in in vitro studies show treatments of omega-3 or omega-6 PUFA, rather than a ratio of both, as seen in a human diet. Treating with only one PUFA or the other is not an accurate representation to what is found in vivo, since a diet must consist of a ratio of omega-3/omega-6 PUFAs, and not a diet consisting of one or the other.

Docosahexaenoic acid (DHA) is one of the most widely studied omega-3 PUFAs in research and is found in foods such as fish oil, salmon, and algae. DHA has also been shown to inhibit a variety of cancers (7, 10, 15). Previous studies have shown that DHA is more potent than other omega-3 fatty acids such as Eicosapentaenoic Acid (EPA). Cancer cells treated with DHASCO, composed strictly DHA, decreased tumor growth more than with Menhaden oil, composed of EPA and DHA (7). Linoleic acid (LA) is an omega-6 PUFA that is found in vegetable oil and processed meats. Many studies have shown that LA promotes tumor growth, and can be dangerous at high quantities in a long

period of time (7). To better represent the average human diet, the current study used treatment of ratios that can be replicated in vivo.

Rather than treat cancer cells solely with DHA, treatments at different ratios of omega-3/omega-6 were used to replicate an actual diet. Currently, the modem western diet consists of an omega-3/omega-6 ratio of about 1:16, while the Japanese diet is about 1:4 (11, 12, 13, 16). As expected, the incidence of cancer in Japan is substantially lower than in the United States (16). This study will show how altering the ratio of fats can have an effect on cancer, but unlike other research, this study can be applied to a human diet. In vivo study has been done on nude athymic mice to see the effects of human diet on prostate cancer, which showed that tumor growth was significantly decreased in human diet ratios (8). In this study, six different ratios of omega-3/omega-6 ratio were observed. These ratios represented common diets found in regular diets and also ratio consisting of high omega-3s.

The ratios were tested on a prostate cancer cell, PC-3 human prostate adenocarcinoma cell. Prostate cancer is the most commonly diagnosed cancer in men. In 2015, there is expected to be 30,000 deaths due to prostate cancer (2). Of the prostate cancer, PC-3 is an aggressive hormone refractory cancer cell line. Research has shown omega-6 PUFAs inhibit the growth of prostate cancer (6, 14). However, a sub cohort study has shown omega-3 PUFAs increases the risk of prostate cancer (4). The goal of this research is to see the effects of the combination of omega-6 and omega-3 PUFAs if they can inhibit prostate cancer growth.

Methods:

Cell lines and Reagents:

PC-3 human prostate adenocarcinoma cell line was used in this study. The two fatty acids, linoleic acid (LA; C18:2 n-6) and docosahexaenoic acid (DHA; C22:6 n-3), were purchased from Sigma Aldrich (St. Louis, MO).

Cell Culture:

The PC-3 cells were cultured in RPMI-1640(Thermo Scientific, Rochester, NY) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT).

Experimental Design:

LA: DHA Ratio	LA:DHA µM Ratio
8:1	88.8 µM:11.1 µM
4:1	80 µM:20 µM
2:1	66.6 µM:33.3 µM
1:1	50 µM:50 µM
3:4	42.9 µM:57.12 µM
1:2	33.3 µM:66.6 µM

Table 1: Experimental design. Total of 100 µM concentration of PUFAs were used.

ATP Assay

Cell viability was done using CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). A white clear-bottom 96-well plates were used, in which, cells were plated and cultured for 72 hour period with respective treatments. At the end of the 72 hour period, the CellTiter-Glo Luminescent assay reagent was added. The

luminescence was measured using SpectraMax M5 plate reader (Molecular Devices LLC, Sunnyvale, CA).

Trypan Blue Assay

Trypan blue stain was also used to check cell viability. Cells were plated on 6-well plates and cultured for 72 hour period along with its respective treatments. Each cells were individually counted with a hemocytometer at the end of the 72 hour period.

Results:

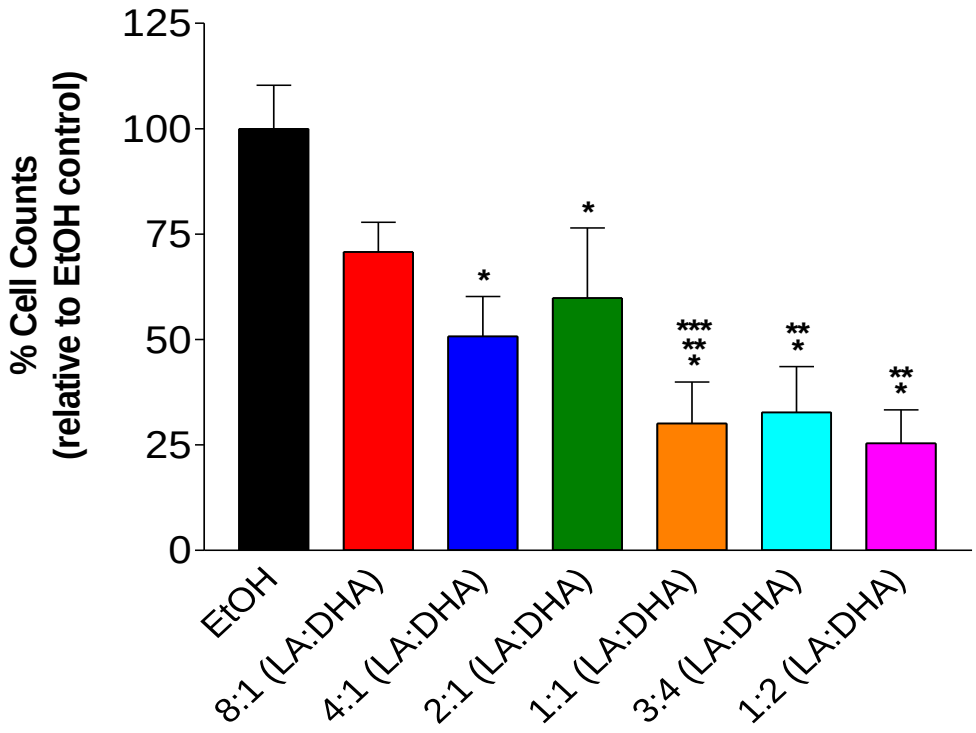

Figure 1. Determining the optimal inhibitory n-6:n-3 ratio in vitro. Hormone refractory prostate PC-3 cells were seeded 24 hours prior to treatment with 100 µM of PUFA at varying LA:DHA ratios. After 48 hours, viable cells were manually counted with Trypan Blue Solution (Mediatech Inc, Manassas, VA). * P<0.05 vs EtOH Control; ** P<0.05 vs 8:1 (LA:DHA); *** P<0.05 vs 2:1 (LA:DHA). *Data represents n=3 independent experiments. Abbreviations: LA (linoleic acid), DHA (docosahexaenoic acid), EtOH (ethanol)*

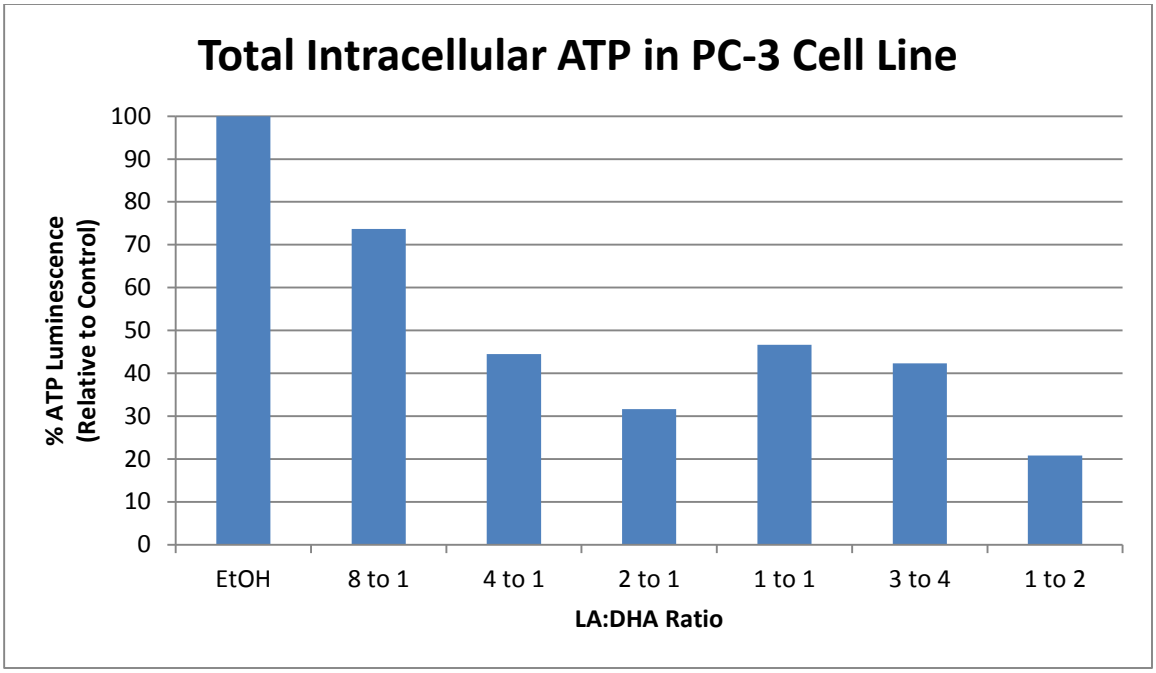

Figure 2. Determining the optimal inhibitory n-6:n-3 ratio in vitro. Hormone refractory prostate PC-3 cells were seeded 24 hours prior to treatment with 100 µM of PUFA at varying LA:DHA ratios. After 48 hours, Promega ATP Assay was used to look at cell viability.

Trypan Blue Assay

Trypan blue assay was done using a cell well plate, in which, each individual cells were manually counted. This assay, as well as the ATP assay, were performed as described by previous research (9). The PC-3 cells showed an increasing inhibition as the ratio of omega-6 to omega-3 PUFAs decreased (Figure 1). The statistical analysis are shown in the figure. The most effective ratio was found to be 1:2, which is the lowest ratio tested.

ATP Assay:

ATP assay was performed using CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). The graph shows a similar pattern as the Trypan Blue Assay (Figure 2). The most effect ratio was found to be 1:2.

Discussion:

Numerous studies have shown the beneficial effects of omega-3 PUFAs. Particularly DHA has been most effective at inhibit cancer as shown in our lab (7). Prostate cancer is the most diagnosed cancer that can be very difficult to treat. Researchers have found that omega-3 PUFAs can be a potential therapeutic treatment in treating aggressive cell lines (6, 14). Our data stays consistent with these findings. Based on the results, increasing concentration of omega-3s inhibits prostate cancer growth. Most importantly, this data disagrees with the sub cohort study that omega-3 increases the risk of prostate cancer (4). In addition, as the omega-6 to omega-3 ratio decreases, the greater the inhibition of cancer. The ratio 1:2 was the most effective at inhibiting growth. It had the lowest amount of prostate cancer cell numbers. However, 1:2 ratio is very unlikely to obtain in a real human diet. The most optimal diet to inhibit the greatest is 4:1 based on the data (Figure 1). One of the lowest ratio found currently worldwide is around 2:1 (12, 13). However, our lab has shown that ratio of 1:1 was possible for the treatment of malignant fibrous histiocytoma of the lungs, in which, the patient took supplements of omega-3 (10).

Based on these finding, having a balance diet of low omega-6 and high omega-3 content may prove beneficial in reducing the risk of cancer. However, this study was a tissue culture study. There may be different result if the same research is done in vivo. Although, in vivo data on nude athymic mice has shown that decreasing the omega-6 to omega-3 ratio does in fact reduces prostate cancer growth (8). Therefore, researching for the possibility of bringing omega-3 fatty acid as a potential therapeutic treatment of cancer may be a valid treatment.

More research can be performed after this study. Now that balance of diet is found to be important in inhibiting cancer, more can be studied to see if this diet can work synergistically with drugs such as chemotherapeutic drugs. Taxotere, a chemotherapeutic drug, was found to be enhanced with omega-3 (8). A following research can be done where it looks to see having a balanced nutrition of fatty acid content can enhance its effect. By enhancing its effect, less amount of the drug can be used. This can have a beneficial side effect. Not only is the drug more effective, but also the patient can take less amount of the drug. This can lead to less side effects and less pain the patient needs to endure. This is one of many direction where this study can be branched into.

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