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The Effects of Omega-3 Fatty Acids on the Concentration of Myo-inositol

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Honors Thesis

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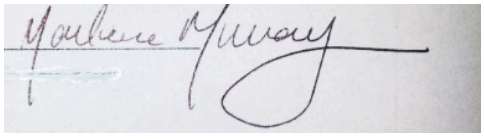
Primary Advisor Signature: 

Full Title: The Effect of Omega- 3 Fatty Acids on the Concentration of Myo-Inositol

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Abstract:

Bipolar affective disorder is a mental illness that affects 1-2% of the population. One hypothesis for the effectiveness of current treatments of bipolar disorder is the reduction of cellular myo-inositol. Lithium and valproate (VPA) are known to reduce cellular inositol levels, however these treatments often have notable side effects. Currently, there is a need to find a treatment for bipolar disorder that reduces the adverse side effects of these drugs. Previous studies, have found success in using omega-3 fatty acids for the treatment of bipolar affective disorder. The long-term purpose of this study is to determine what effect omega-3 fatty acids have on myo-inositol concentration. This study has worked toward this purpose by comparing the growth effects of 0.8mM DHA to 2.5mM VPA. We have found that similar to VPA, cell growth is reduced at 0.8mM.

Keywords: valproate, bipolar disorder, myo-insitol, omega-3 fatty acids

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Introduction:

Bipolar affective disorder is a mental illness that affects 1-2% of the world population according to a study by the World Health Organization in 1996 (Ding, Shi, Shaltiel, 2009). Bipolar disorder is characterized by severe bouts of mania and depression. Current market drugs Lithium, Valproate (VPA), and Carbamazepine have proven affective mood stabilizers. The initial effect of these drugs has been the reduction of intracellular myo-inositol (Ding, Shi, Shaltiel, 2009). Further studies have shown, that the size of sensory neuronal growth cones react in an inositol dependent manner (Ding, Shi, Shaltiel, 2009). Although these current market drugs have proven affective mood stabilizers, they often have adverse side affects. For example, Lithium causes polyuria, sedation, and weight gain while valproate has been shown to trigger weight gain, sedation, nausea, and hair loss among some patients participating in clinical trials (Stoll, Locke, Marangell, 1999). In consideration of these adverse side effects, there is a need for effective mood stabilizers that avoid these adverse effects.

Fatty acids including Omega-3 fatty acids have been shown to alleviate some of the symptoms of bipolar disorder (Stoll, Locke, Marangell, 1999). Omega-3 fatty acids are less toxic than the previously mentioned market drugs and the only adverse effect known has been mild gastrointestinal irritation. As previously mentioned, current market drugs VPA, lithium, and carbamazepine have been shown to reduce intracellular myo-inositol.

Although there exists no direct evidence linking myo-inositol depletion to the mood stabilizing effect of current market drugs, studies have shown that myo-inositol decreased sensory neuronal growth cones. When these growth cones were treated with current market drugs lithium and valproate they increased sensory neuronal growth cones, upon a increase in the concentration of myo-inositol sensory neuronal growth cones decreased again which suggests their antagonistic nature (Harwood, 2004). Inositol levels in bipolar patients are altered in normal individuals under the influence of these market drugs and they could be working to stabilize inositol levels (Harwood, 2004). In consideration that omega-3 fatty acids have had a similar therapeutic function, the purpose of this research has been to determine the effect of Omega-3 fatty acids, specifically docahexanoic acid (DHA), on the concentration of myo-inositol. The effect on concentration was determined by comparison of valproate at the concentration of 2.5millimolar and DHA at 0.8mM. This effect was observed on the model system *Saccharomyces cervisiea,* because they have been shown to have similar components of the myo-inositol signaling pathway as observed in humans (Ding, Shi, Shaltiel, 2009). This study indirectly measured inositol concentration by observing its effect on the proliferation of Saccharomyces *cervisiea* in the absence of added inositol.

Materials and Methods:

Growth of Yeast Cells

Yeast cells were placed in 100ml of media and grown overnight for 20 hours at 30°C with constant shaking. The concentration of the overnight culture was then determined spectrophotometrically. To accomplish this a 1:10 dilution of the cell culture was made by mixing 100 microliters of the overnight culture in 900 microliters of sterile broth (media) and placing the mixture in a clean cuvette. The optical density obtained is equivalent to the concentration of the culture. The program used to determine the optical density is Softmax Pro. Based on the determined concentration, an appropriate volume of the overnight culture was used to inoculate three experimental cultures to an optical density of 0.1. The three experimental cultures consisted of 300 milliliters of media, one containing 2.5millimolar of valproate, another containing 0.8millimolar of DHA and a third lacking both drug and DHA was used as control. The experimental cultures were allowed to grow for 24 hours at 30°C with shaking.

Determine the Effect of Omega-3 and Valproate on Growth

Serial dilutions of the experimental cultures were performed by adding 10 microliters of each culture in 990 microliters of sterile broth media in a sterile microcentifuge tube and then mixing thoroughly. Then 10 microliters of this solution was diluted further in 990 microliters of sterile broth media, in another microcentrifuge tube with through mixing. Finally, 100 microliters of this dilution was spread evenly on a YPD plate with a sterile spreader and allowed to incubate at 30°C for three days. The effects of the DHA and VPA was determined by counting the number of colonies on each plate.

Results:

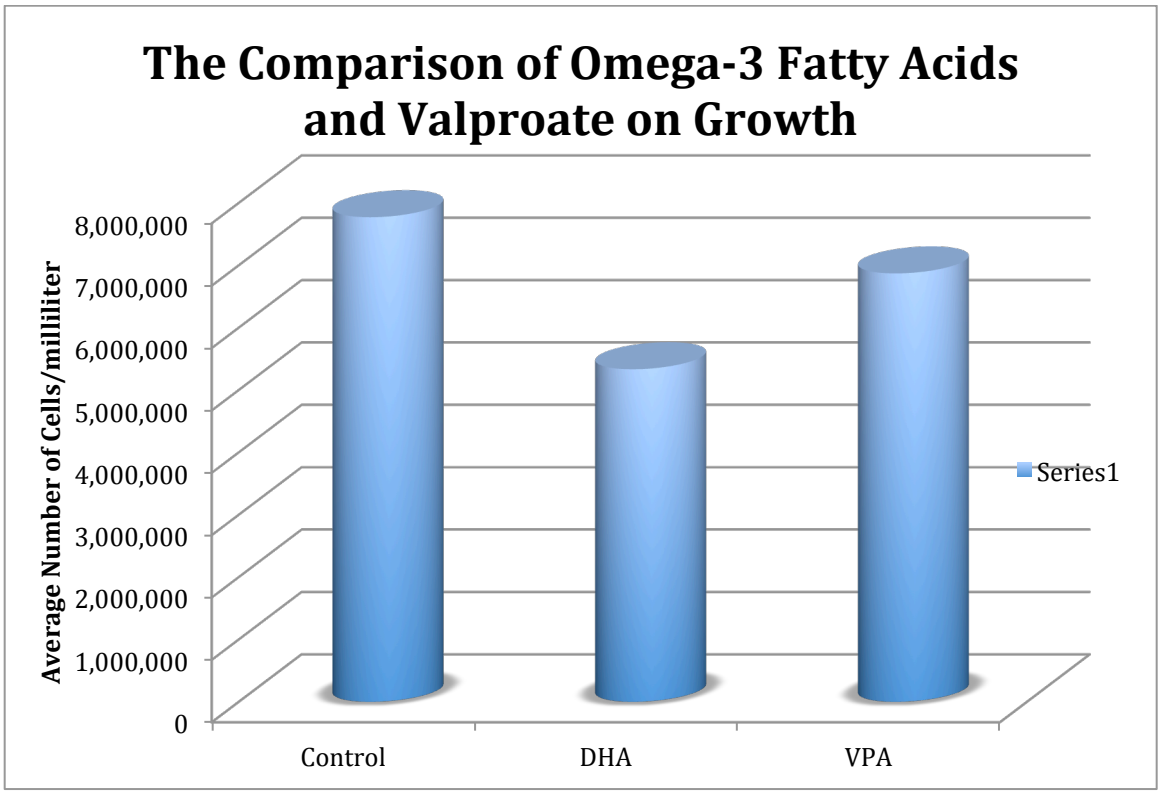

Figure: 1
The average number of cells grown in 0.8mM DHA, (SEM=**5333320**)2.5mM VPA (SEM=**6865788**), and Control (SEM=**7765658**). *n*=3

Cell Growth Trial#1

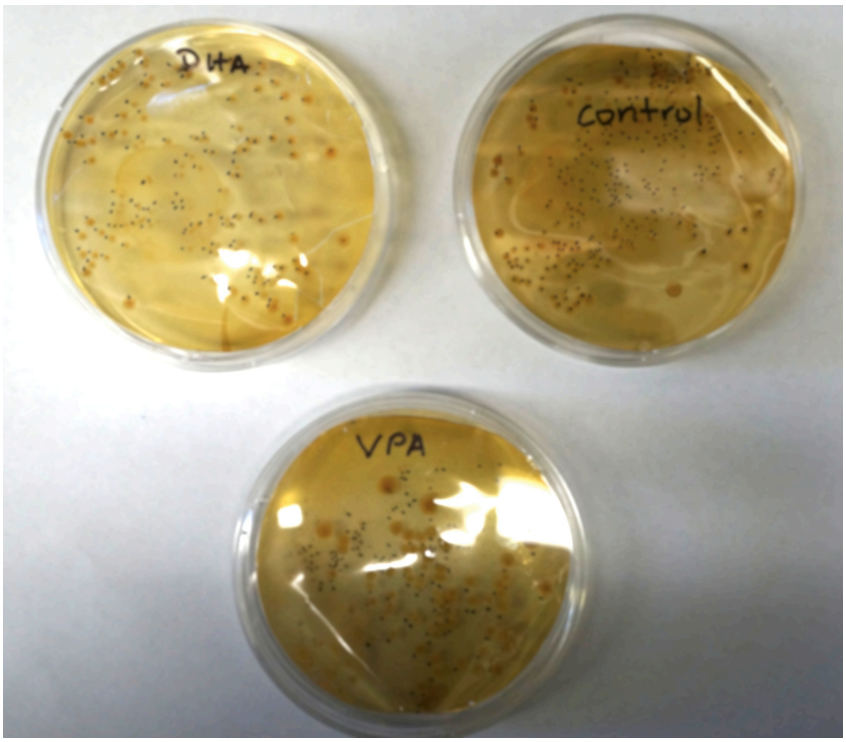

Figure: 2
Representative of Cell Growth

As indicated in figure 1, the average of the three trials showed that 7770749 cells grew among the control, 5333383 cells grew under the influence of DHA, and 6870178 cells grew under the influence of VPA. Figure 1 indicated that DHA was most inhibiting to cell growth. Furthermore, upon statistical analysis via a one-way ANOVA a p-value of 0.00746 was obtained.

Discussion/ Conclusion:

In this study we compared the effects of DHA and VPA on yeast cell growth. We know that VPA inhibits cell growth and intracellular myo-inositol. If found that DHA inhibits cell growth, then it is possible that it also reduces cellular myo-inositol. In comparison to the control cell count numbers, DHA has been shown to inhibit cell growth, resulting in fewer cells at 0.8 millimolar. Furthermore, it has been shown to be more inhibitory than VPA at 2.5millimolar, producing on average 5,333,383 cells in comparison to VPA producing 6870178 cells. Obtaining a p-value of 0.00746, much less than 0.05, I fail to reject the null hypothesis that there is no difference between either experimental cultures or the control. Therefore, the any differences between control and experimental cultures, VPA and DHA data are statistically significant. The data suggests that similar to VPA, DHA at 0.8 micromolar is highly inhibiting therefore the follow up to this work is to directly measure the effects of 0.8 millimolar DHA on inositol concentration,

Acknowledgements:

Special thanks to Dr. Marlene Murray for guiding me through this project with insightful critiques and words of wisdom, her work in the administration of anti-bipolar medication are models of stellar scientific quality.

Annotated Bibliography:

Ashizawa, Naoki, Motoyuki Yoshida, and Tomoji Aotsuka. "An enzymatic assay for myo-inositol in tissue samples." *Journal of Biochemical and Biophysical Methods* 44.1-2 (2000): 89-94. Print.

This article by Ashizawa describes the process of enzymatically measuring the concentration of myo-inositol in sciatic nerve and lens of diabetic rats. The article is helpful as it illustrates a biochemical method by which the concentration of myo-inositol can be directly measured for continued research on this subject. A diagram for the NAD+ coupled reaction is presented in the article that describes how myo-inositol is related to formazan that can spectroscopically be measured.

Ding, D., Shi, Y., Shaltiel, G., Azab, A. N., Pullumbi, E., Campbell, A., et al. (2009). Yeast bioassay for identification of inositol depleting compounds. *World Journal of Biological Psychiatry*, *10*(4_3), 893-899.

Yeast Bioassay for Identification of Inositol Depleting Compounds provided a description of the characteristics of bipolar mood disorder, including how many people are affected by the disorder in the United States as well as its global impact. Furthermore, this article explained specifically how valproate reduced cellular inositol levels and shed some light on other mood stabilizing medication, namely carbamazepine. This article also demonstrated how growth cones operate in neurons and how valproate may actually target myo-inositol-3-phosphate synthase the enzyme responsible for synthesis of myo-inositol. I will use this article's definition of bipolar mood disorder and it detailed description of the effects of VPA when comparing it to find out if omega-3 fatty acids reduce cellular inositol levels by the same mechanism.

Harwood, A. J. (2004). Lithium and bipolar mood disorder: the inositol-depletion hypothesis revisited. *Molecular Psychiatry*, *10*(1), 117-126.

Lithium and Bipolar Mood Disorder: The Inositol-Depletion Hypothesis Revisited described the characteristics of myo-inositol and how it functions in the human body. The article provided more information on what is known about how lithium and valproate and how they interact with the myo-inositol pathway. Information on inositol uptake molecules SMIT and HMIT was also given in addition to a description of how they are active within specific pH ranges. This research suggests further avenues by which the decrease in myo-inositol concentration due to the influence of omega-3 fatty acids might be explored.

Stoll, A., Locke, C., Marangell, L., & Severus, W. (1999). Omega-3 fatty acids and bipolar disorder: a review. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *60*(5-6), 329-337.

Locke and Marangell's article, *Omega-3 Fatty Acids and Bipolar Disorder: A Review*, provided a table of all the common mood stabilizing drugs including omega- 3 fatty acids in addition to information about each drugs efficacy, common side effects, toxicity, and any other important information. This article gave a very through description of the structure of omega-3 fatty acids and it's relative abundance. The article further emphasized why omega-3 fatty acids are a potential solution to mood regulation among bipolar disorder sufferers.