Andrews University
Digital Commons @ Andrews University

Honors Theses | Undergraduate Research

2011

The Effects of Omega-3 Fatty Acids on Saccharomyces Cerevisiae Inositol Pathway Mutants

Daniel Antonio Colon Hidalgo

Marlene Murray - Nseula

This research is a product of the graduate program in Biology at Andrews University. Find out more about the program.

Follow this and additional works at: https://digitalcommons.andrews.edu/honors

 Part of the Biology Commons

Recommended Citation

Colon Hidalgo, Daniel Antonio and Murray - Nseula, Marlene, "The Effects of Omega-3 Fatty Acids on Saccharomyces Cerevisiae Inositol Pathway Mutants" (2011). *Honors Theses*. 11.
https://digitalcommons.andrews.edu/honors/11

This Honors Thesis is brought to you for free and open access by the Undergraduate Research at Digital Commons @ Andrews University. It has been accepted for inclusion in Honors Theses by an authorized administrator of Digital Commons @ Andrews University. For more information, please contact repository@andrews.edu.

Andrews University
Seek Knowledge. Affirm Faith. Change the World.

Thank you for your interest in the

Andrews University Digital Library

Please honor the copyright of this document by not duplicating or distributing additional copies in any form without the author's express written permission. Thanks for your cooperation.

The Effects of Omega-3-Fatty Acids on *Saccharomyces Cerevisiae* Inositol Pathway Mutants

Colon, Daniel and Murray-Nseula, Marlene
Department of Biology, Andrews University, Berrien Springs, Michigan 49104

ABSTRACT

Bipolar disease is a severe and chronic debilitating mental disorder affecting 1-3% of the population. Omega-3-fatty acids have been shown to relieve symptoms of bipolar disorder and are not associated with the negative side effects of lithium and valproate, two of the commonly used drugs for treating this condition. The mechanism of action of omega-3-fatty acids remains unknown. Therefore the purpose of this study was to determine if, like lithium and valproate, omega-3-fatty acids affect inositol metabolism. This was accomplished by investigating the effect of the omega-3-fatty acid docosahexanoic acid (DHA) on the growth of inositol pathway mutants. To measure the effects of DHA we grew an inositol auxotrophic mutant (SH1) and an inositol overproducing mutant (SH7) in the presence and absence of DHA and compared the growth. The results were similar to previous studies done with valproate, showing that in the absence of DHA, growth of the inositol overproducer is better than the growth of the inositol auxotroph due to the abundance of inositol in the overproducing mutant. The results also show that DHA inhibits growth of all strains including the inositol overproducing strain. Therefore, it may be concluded DHA has an effect on the inositol pathway since the decrease in growth of the overproducing strain may be due to a decrease in the inositol levels of the overproducing strain by DHA.

Introduction

Bipolar disorder, also known as manic-depressive illness is a mental condition that causes very severe changes in a person's mood, which can go from a very excited state to a depressive state in a short period of time. Statistics show that around 1.5% of the United States population currently suffer from this illness. Although there are some treatments, there is not a known cure for bipolar disorder. One of the most common treatments of bipolar disease is lithium, which was approved in the 1970s by the FDA as a treatment for this illness. Also in 1995, the FDA approved valproic acid as another treatment for this condition.

Even though that these drugs are quite common and have helped some of the patients treated, they are far from being perfect because they are not always effective and in many cases cause some negative side effects. In the case of lithium, some patients treated with it have suffered problems with their thyroid gland. For example, hypothyroidism, which can result in weight gain, has been seen in some patients that have been exposed to lithium during their treatment (Henry 2002). Also, another effect that has been associated with lithium treatment is tremors (Gelenberg et al., 1995). Other neurological effects that are associated with the lithium treatment include seizures, extrapyramidal effects, changes in electroencephalogram (EEG), dizziness, speech problems and gastrointestinal effects like nausea and diarrhea (Ghadirian et al., 1980).

Valproic acid has also been associated with several different side effects, although not as severe as the ones associated with the use of lithium. Some of the side effects of the use of valproic acid include: weakness, facial edema, vomiting, abdominal pain, nausea, vomiting, nosebleed, blood clotting problems and lethargy (Kulick et al., 1993).

Since the current treatments used to treat bipolar disorder are associated with negative side effects there is a need for a better treatment for bipolar disorder. In order to start developing better treatments, the molecular mechanism of the action of lithium and valproic acid has been the subject of different research projects in the past few years. Studies have shown some relationship between lithium and the inositol biosynthetic pathway. Lithium inhibits myo-1-inositol monophosphatase, an enzyme whose function is involved in the recycling of inositol (Murray and Greenberg, 1999; Parthasarathy 1997). Also valproic acid has been shown to affect the inositol biosynthetic pathway by inhibiting an enzyme called myo-inositol phosphate synthase which catalyzes the conversion of glucose-1-phosphate to myo-inositol-1-phosphate (Shaltiel et.al 2004). Both drugs have been shown to decrease intracellular inositol levels (Vaden et. al., 2001).

Research has shown that omega-3-fatty acids relieve some of the symptoms of the patients with bipolar disease (Stoll et al. 1999). In addition, previous studies show that omega-3-fatty acids inhibit phospholipase C (PLC), protein kinase C (PKC) and Inositol-3-phosphate (IP_3), all components of the inositol biosynthetic pathway (Mirnikjoo et al., 2001).

In preliminary work done in Dr. Murray-Nseula's lab, it was shown that the omega-3-fatty acid docosahexanoic acid (DHA) affects the growth of the yeast *Saccharomyces cerevisiae* and that this inhibition is reversed in the presence of inositol. To further investigate the effect of omega-3-fatty acids on the inositol biosynthetic pathway, in this study, I will use *Saccharomyces cerevisiae* strains that have mutations in the inositol biosynthetic pathway that cause them to either overproduce inositol (*opi1*) or to not be able to produce inositol (*ino1*). These strains were grown in the presence or absence of DHA to determine if the inositol overproducing phenotype is more resistant to growth inhibition by DHA and if the inositol auxotrophic phenotype is more

sensitive to DHA. The purpose of this project is to test the effect of omega-3-fatty acids on the inositol biosynthetic pathway. My null hypothesis is that the addition of DHA does not affect the inositol levels on the yeast cells, hence the addition of DHA does not affect the growth of the yeast cells.

Materials and Methods

Thawing yeast strains

For this project three different strains of the yeast *Saccharomyces cerevisiae* were used: an inositol auxotroph- that does not produce inositol (SH1), an overproducer (SH7) and a wildtype (SMY7). These strains were kept immortalized in microcentrifuge tubes in a freezer set to -80^{o}C. A sample of each of the strains was taken using a sterile toothpick and was plated on YPD plates and they were incubated for 72 hours at a temperature of approximately 30^{o}C.

Growth Media

Two different types of media were made for the different strains' needs (the media was made using a recipe provided by instructor). One of the media lacked inositol (I^{-}) and was used to grow the overproducer and the wildtype strains. The other type of media contained inositol in a 10 μm concentration. This was done because the inositol auxotrophic strain needed some inositol to be able to grow.

Growth Conditions

After the 3 different thawed strains were grown for the 72 hours, a small sample from each of the plates was put into a flask containing 100 ml of the media for each straing, using a sterile toothpick. . The cultures were then grown in a shaking water bath overnight (18 hours) at

150rpm and 30 ^{o}C. Once the 18 hours were done an optical density reading of each of the cultures was done. In order to do this, 100 μl of each of the cultures were diluted with 900 μl of media.

Samples of the overnight cultures were placed in the experimental media containing or lacking DHA^{-} The amount of each culture used was dependent on the optical density reading.; the formula $M_1V_1=M_2V_2$ was used (M_1= optical density reading, M_2= desired concentration of .1, V_1 was the amount of culture that was needed to be put into the flask that was going to have DHA and V_2= 100mL of media). The experimental cultures were taken to grow for 24 hours in a shaking water bath at 150 rpm and 30^{o}C.

Colony plating and counting

After the 24 hours,, an optical density reading of each of the culture was taken, 100 μl a 10^{-5} serial dilution was plated on YPD plates and incubated for 72 hours, at which time colonies were counted.

Results

Table 1.1 Total number of colonies.

	1		2		3		4	
Strain	DHA+	DHA-	DHA+	DHA-	DHA+	DHA-	DHA+	DHA-
SH1	122	146	173	608	161	231	123	365
SH7	147	399	255	950	191	408	207	513
Wildtype	0	0	262	297	72	203	139	214

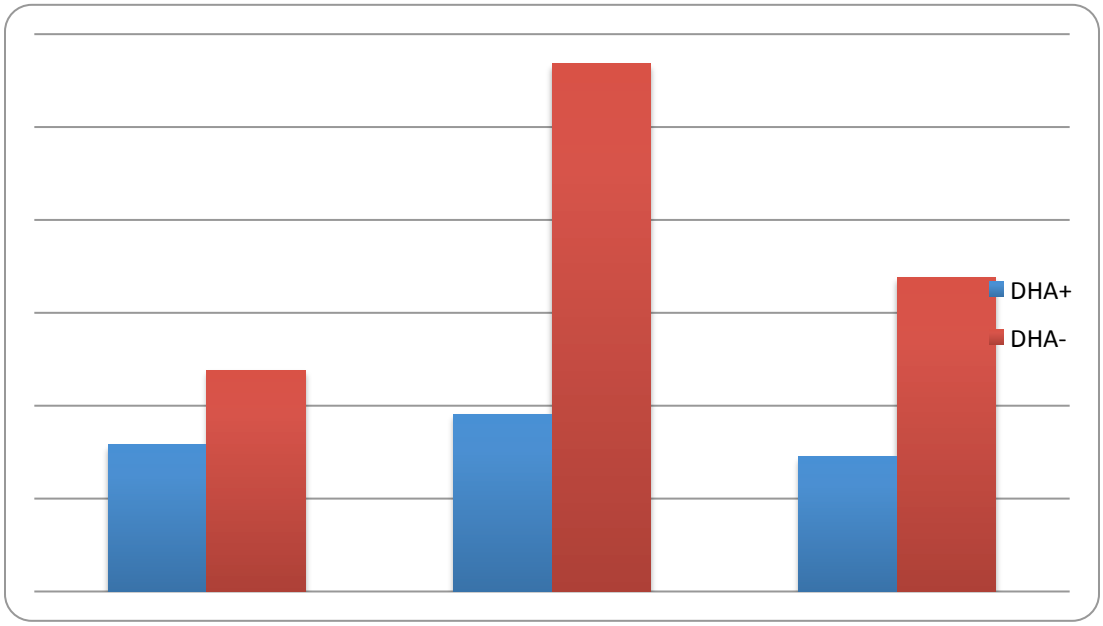
The table represents the results of four separate trials. It shows the number of colonies counted on each of the runs for each of the strain in the presence and absence of DHA.
The wildtype strain used in this experiment did not grow as expected during the first run, so first run of the experiment was completed with only the SH1 and SH7 strains.

Table 2.1 Average number of colonies

Strain	DHA+	DHA -
SH1	145	338
SH7	191	568
Wildtype	158	238

Table 2.1 contains the average number of colonies grown of each of the strains in the presence and absence of DHA.

Figure 1.1 Graphical representation of average number of colonies



Discussion

What we expected to find as we grew the different strains in the presence and absence of DHA, was that the number of colonies should have decreased when the DHA was present in all of the strains. From the data it can be seen that when the DHA was added the growth of cells decreased significantly in the case of the SH1 strain ($\chi^2 = 6.79$, $df = 2$, $p < 0.01$) and the SH7 strain ($\chi^2 = 7.84$, $df = 2$, $p < 0.01$), which supports our expectations for these 2 strains. In the case of the wildtype our data is not as significant as we would have expected. The fact that our data for the wildtype strain was not significant was probably because our sample size was not large enough since in the first run of the experiment we did not get any growth of these type of cells, the acquisition of more data could have made our results more concrete and statistically significant.

Also, it was observed that the overproducer strain grew better than both the wildtype and the inositol auxotroph in the presence of DHA. This was as expected since in the overproducer strain there were larger concentrations of inositol, which promotes the growing of the colonies. However, an important detail is that when DHA was added, the growth of all the colonies decreased to about the same level, which suggests that the decrease in the growth is due to the decrease in inositol levels.

Another interesting observation was that in the absence of DHA the SH1 strain grew better than the wildtype. We expected the contrary since the wildtype was able to produce its own inositol and the SH1 strain was not. The results obtained might have been due to the fact that we added 10 μm inositol to the media in which the SH1 grew thus creating an environment containing more inositol than that produced by the wildtype.

Although the results support our expectation, there are other studies that could be done in order to support the data gotten in this research project. For example, the actual inositol concentration levels could be measured by spectrophotometry in cells grown in the presence or absence of DHA.

References

Chen, G., L.D. Huang, Y.M. Yiang and H.K. Manji. 2000. The mood-stabilizing agent valproate inhibits the activity of Glycogen Synthase Kinase-3. Journal of Neurochemistry **72**: 1327-1330.

Gelenberg, AJ., J.W. Jefferson. 1995. Lithium tremor. Journal of Clinical Psychiatry **56**: 283-287.

Ghadirian, A., H.E. Lehmann. 1980. Neurological side effects of lithium: Organic brain syndrome, seizures, extrapyramidal side effects, and EEG changes. Comprehensive Psychiatry **21**: 327-335.

Henry, C. 2002. Lithium side-effects and predictors of hypothyroidism in patients with bipolar disorder: sex differences. Journal of Psychiatry and Neuroscience **27**: 104-107.

Kulick S.K., D.A. Kramer. 1993. Hyperammonemia secondary to valproic acid as a cause of lethargy in a postictal patient. Annals of Emergency Medicine **23**: 904-905.

Mirnikjoo, B., H.F. Kim, L.B. Marangell, J.D. Sweatt and E.J. Weeber. 2001. Protein kinase inhibition by omega-3 fatty acids. Journal of Biological Chemistry **276**: 10888-10896.

Murray, M., M.L. Greenberg. 1997. Regulation of Inositol Monophosphatase in the yeast *Saccharomyces cerevisiae*. Molecular Microbiology **25**: 541-546.

Parthasarathy, L. 1997. Molecular characterization of coding and untranslated regions of cortex lithium-sensitive myo-inositol Monophosphatase cDNA. Gene **191**: 81-87.

Shaltiel, G., A. Shamir, D. Ding, E. Dalton, M. Bailer, A.J. Harwood, R.H. Belmaker, M.L. Greenberg and G. Agam. 2004. Valproate decreases inositol biosynthesis. Biological Psychiatry **56**: 868-874.

Stoll, A., W.E. Severus, M.P. Freeman, S.Rueter, H.A. Zboyan, E.Diamond, K.K. Cress, L.B. Marangell. 1999. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo controlled trial. Archives of General Psychiatry **56**: 407-412.

Vaden, D., D. Ding, B. Peterson, M.L. Greenberg. 2001. Lithium and Valproate decrease Inositol mass and increase expression of the yeast *INO1* and *INO2* genes for Inositol biosynthesis. Journal of Biological Chemistry **276**: 15466-15471.