THE ROLE OF 5-AMINOLEVULINIC ACID (5-ALA) AND SLEEP

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The study food supplement was provided by the sponsor. Investigators and authors had full control over the studies. There is no conflict of interest between the Investigator and SBI Pharmaceuticals Co. Ltd. The formulation used in the University of Hawaii studies is available in Japan as a food supplement.

ABSTRACT

Objective: To determine if there is a relationship between the administration of the dietary supplement containing 5-Aminolevulinic Acid (5-ALA) and sleep.

Methods: A double-blind, randomized parallel-group study was conducted. It was a 4 month study of 40 participants between the ages of 40 and 70. Males and females were recruited equally. There were 20 in each group who had existing sleep disorders, excluding sleep apnea and Restless Leg Syndrome (RLS). The tools used to measure participant sleep improvement included: the Body Mass Index (BMI - a measure of body fat based on height and weight), a daily diary and the Pittsburgh Insomnia Rating Scale–20 Question (PIRS-20). The PIRS-20 design suggests improved sleep when the total score is lower.

Results: Improvement in sleep in the group taking 50 mg 5-ALA, compared to controls, was significant. The mean change, from baseline through week 6, was -5.67 units less on the sleep scale than the control group with a p value of .001. The mean change from week 6 to week 10 when the participant was no longer taking the supplement was 4.55 units higher than the control with a p value of .062, which is of borderline significance.

Conclusion: There appears to be a relationship between the administration of dietary supplements containing 5-ALA and sleep. The results of this study suggest that 5-ALA does in fact improve sleep. The mechanism for sleep improvement needs to be explored. Further research is warranted.

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LIST OF ABREVIATIONS

ANOVA	Analysis of Variance
ATP	Adenosine-5'-triphosphate
BCC	Basal Cell Carcinoma
BMI	Body Mass Index
BSE	Bovine Spongiform Encephalopathy
CBC	Complete Blood Count
DLMO	Dim-Light Melatonin Onset
DLS	Diagnostic Laboratory Services
GLP	Good Laboratory Practice
GMO	Genetically Modified Organism
HbA1C	Hemoglobin A1C
OGTT	2 hour post-Oral Glucose Tolerance Test
PDT	Photodynamic Therapy
PIRS-20	Pittsburgh Insomnia Rating Scale–20 Question
PRC	Phase Response Curve
RLS	Restless Leg Syndrome
SBI	Strategic Business Innovator
SCC	Squamous Cell Carcinoma
SCN	Suprachiasmatic Nucleus
SFC	Sodium Ferrous Citrate
SSS	Supplement Sleep Study
5-ALA	5-Aminolevulinic Acid

CHAPTER 1. INTRODUCTION

5-Aminolevulinic Acid (5-ALA) is a dietary supplement. Five-ALA can be found in many common foods (see Table 1). The production methods are described along with a brief history of research done on this amino acid in this section. The potential relationship between 5-ALA and improved sleep and its mode of action will then be described. Studies that address the preclinical and safety data for the use of 5-ALA as an oral supplement are also provided. The purpose of this investigation is to determine if a relationship exists between the administration of dietary supplements containing 5-ALA and sleep.

The structure of 5-ALA is described in Figure 1. In the prophyrin synthesis pathway, 5-ALA is the first compound. In mammals, the prophyrin synthesis pathway leads to the synthesis of heme and in plants, chlorophyll is synthesized. Plants that are fed 5-ALA can accumulate toxic amounts of the chlorophyll precursor, protochlorophyllide, indicating that the synthesis of this intermediate is not suppressed anywhere downstream in the pathway. 5-ALA is produced in non-photosynthetic eukaryotes such as animals, insects, fungi and protozoa; also, the α-proteobacteria group of bacteria. It is produced by the enzyme ALA synthase, from glycine and succinyl CoA. This reaction is known as the Shemin pathway.[1]

In plants, algae, bacteria (except for the α-proteobacteria group) and archaea, 5-ALA is produced from glutamic acid via glutamyl-tRNA and glutamate-1-semialdehyde. The enzymes involved in this pathway are

glutamyl-tRNA synthease, glutamyl-tRNA, glutamyl-tRNA and glutamate-1-semialdehyde aminotransferase. This pathway is known as the C5 or Beale pathway.[2, 3]

5-ALA elicits synthesis and accumulation of fluorescent porphyrins (protoporphyrin IX) in neoplastic and epithelia tissues, among them malignant gliomas. In neurosurgical procedures, 5-ALA is used to visualize tumorous tissue. Studies suggest that the continual use of this particular visualization method may reduce the tumor residual volume and prolong progression-free survival in patients suffering from malignant gliomas.[4] It is also used as a photosensitizer for photodynamic therapy.

Cancer Treatment Photodynamic Therapy is a non-conventional light therapy for the treatment of cancer. Photodynamic therapy, also known as PDT, uses photosensitive drugs (5-ALA, Foscan, Metvix, Tookad, WST09, WST11, Photofrin and Visudyne) which are triggered by light from a specific wavelength, usually red or infrared, on the light spectrum chart.[4] Depending on the type of cancer being treated, the medications are administered differently. A singlet oxygen molecule will form when the light is applied to the drug, this molecule attacks the tumor and destroys it from the inside out. Photodynamic therapy is a proven alternative treatment for many cancers. Clinical trials have taken place worldwide and several hospitals offer PDT as a primary cancer treatment.[5] This is a high dose therapy. In this protocol a low dose was used. The amount used in cancer therapy is 20 mg/kg, or about 1000 to 1500 mg, a day. In this trial

50 mg were administered (see Table 4). At high doses, phototoxicity has been reported. No phototoxicity, as seen in Table 2, has been reported at low doses which are similar to concentrations found in many common foods.

Photodynamic detection is the use of photosensitive drugs with a light source of the right wavelength to detect cancer. Treatment possibilities include – prostate cancer, breast cancer, Giant Basal Cell Carcinoma (BCC) (Skin), cervical cancer, recurrent bladder cancer, vulvar cancer, brain cancer (human glioblastoma), HPV-induced cancers, colon cancer, leukemia, Barrett's esophagus, lung cancer, stomach cancer, head and neck cancers, squamous cell carcinoma (SCC), Bowen's disease, penile and other types of cancer.

Until recently 5-ALA was difficult and costly to produce. Using 5-ALA to treat cancer was a preferred alternative to ionizing radiation therapy due to the risks associated with radiation exposure. Using 5-ALA for alternative medicine for other treatments was simply not economical.

5-ALA is known to be a basic building block of heme production. The porphyrin synthesis pathway that leads to heme production in mammals is composed of several compounds and 5 ALA is the first. Heme plays a role in cellular energy production.[2] Cellular energy generation uses membrane-localized electron transfer chains for adenosine-5'-triphosphate (ATP) synthesis.[2] ATP uses the cells to transport chemical energy for metabolism.[2] Understanding the relationship between 5-ALA and heme production might provide evidence that explains why 5-ALA has a relationship with sleep.

Production Methods

Recent innovations at SBI Pharmaceuticals Co. Ltd. have made it possible to make this supplement available at a reasonable cost. This new fermentation process has many advantages: a) *production costs* are low, b) *production rates* are increased, and c) *safety and environmental friendliness* are improved, compared with other products.

History of Research on 5-ALA

5-ALA is a natural amino acid. It is also a precursor of chlorophyll or heme. Eight molecules of 5-ALA make a porphyrin. A porphyrin is a very flat molecule; it has many double bonds and nitrogen's lone pair electron. A porphyrin can capture energy, store it and carry it. Photosynthetic bacteria, especially purple-nonsulfur bacteria, accumulate ALA under light illumination in the presence of levulinic acid. The use of light illumination on an industrial scale is not economical.[3]

According to the literature, increased production of glucose in the cell results in decreased 5-ALA production.[6] At the same time, decreased heme production occurs from aging. This results in decreased heme enzyme activity. A decline in the mitochondrial electron transfer system follows, with decreased basal metabolism - as well as physical energy decline and possibly depression.

Preclinical and Safety Literature

Five-ALA has been used for many years at relatively high dose amounts in single doses for photodynamic therapy and immunofluorescence of tumors. An analysis of compiled data relating to safety and any side-effects about of the use of 5-ALA at low doses has not yet been published. The safety of low-dose 5-ALA as a supplement has been demonstrated by animal and human studies. An analysis of the current literature (Medline, SBI and use of the snowballing technique) related to the safety and efficacy of 5-ALA in animals and humans is presented below. Clinical trials using 5-ALA are also discussed.

5-Aminolevulinic Acid is a natural non-alpha amino acid. 5-ALA is a delta amino acid and is not a component of protein. It is found in many common foods such as spinach, tomatoes, mushrooms, potatoes, wine, etc.

5-ALA is synthesized in the mitochondria. It is a building block of protoporphyrin and a precursor of both chlorophyll and heme. The safety of 5-ALA combined with iron in a supplement at low doses has been demonstrated by animal and human studies. 5-ALA has been associated with the origin of life. There are three fundamental components of living organisms:

- Nucleotides → Genetic Information,
- Amino Acids → Structure & Metabolism, and
- 5-ALA → Energy Conversion.

5-ALA Facts

The blood concentration of 5-ALA in normal human beings is approximately 50µg/L. The typical intake from food is approximately 1-2 mg/day. Five-ALA is synthesized by the body at a rate of 600 mg/day.[7] About 1 mg of 5-ALA is in plasma.[8] Normal plasma level in 5-ALA was 92 nmol/l (SD = 39, n = 89 with a range of 24-270 nmol/l).[8]

The internal heme pool has about 60 g in hemoglobin and 8 g in other heme enzymes. Five-ALA is excreted in the urine and as bilirubin at a rate of 2 mg/day. The bioavailability is 100% ($D_{i.v}/D_{p.o}$). The t_{max} in plasma is 0.76 h and the $t_{1/2}$ in plasma is 0.92 h as estimated from a sample of healthy human (18-55y/o) males, N=12, given 20mg/kg of 5-ALA.[8]

The oral 5-ALA product used in all of the safety studies were produced by Cosmo Oil Co. Ltd. The supplement contains 3 components:

1) 5-Aminolevulinic Acid (5-ALA) phosphate salt
2) Citric iron
3) Corn starch (as a filler)

It is a Non-Genetically Modified Organism (GMO), Bovine Spongiform Encephalopathy (BSE) free, alcohol free and the products are manufactured under food Good Laboratory Practice (GLP) conditions. A certificate of analysis is available. No heavy metals in the analyses (Pb, As, Hg, Cd) were detected and a microbial analysis revealed viable bacterial counts < 300/g. Neither E.coli or S.aureus were detected.[3, 9]

Studies Completed

Preclinical and Safety Tests suggest that 5-ALA is safe at modest doses. A summary of these findings from investigations conducted by Cosmo Oil & SBI can be seen in Tables 6 and 7.[9] In these studies, 3 different doses of 5-Aminolevulinic acid (5-ALA) phosphate, Sodium Ferrous Citrate (SFC) and Corn starch as filler were tested in humans. The steps were: 25mg of 5-ALA/29 mg Sodium Ferrous Citrate (SFC) in step1, 50mg 5-ALA /58mg SFC in step 2 and 75mg 5-ALA /87mg SFC in step 3 (See Table 6). Twenty-two participants (11 male and 11 female) in each step (total 66 subjects) took test food supplement for 4 weeks. Participants were healthy adults. This particular investigation used higher dosages than used in the investigations that occurred in Hawaii. The safety of the test food supplement was evaluated by subjective symptoms and the clinical examination of subjects in the intake period .The change of laboratory test data were evaluated by using the one-sample t-test about the mean value of measurements obtained at 2 weeks and 4 weeks after administration, and 2 weeks after the end of the follow-up period, compared to those obtained just before the start of administration.

Adverse effects were noted in 19 subjects (6 subjects in step 1, 4 subjects in step 2, and 9 subjects in step 3). Total numbers of adverse events were 24 (6 events in step 1, 4 events in step 2, and 14 events in step 3). Symptoms in step 1 included diarrhea (2 events), headache (2), abdominal pain (1), facial swelling (1). Symptoms in step 2 included diarrhea (2 events), queasiness (1), stomach pain (1). Symptoms in step 3 included abdominal pain (4 events), rheumatoid

symptoms (4), diarrhea (3), flatulence (2), stiff shoulders (1). All adverse events were self-recognized subjective symptoms and judged by the Investigator to be unrelated to the study diet intake and to be mild in severity. Some data from laboratory test parameters showed statistically significant changes from baseline. All those changes (mild nausea, headache and GI upset) were relatively mild in severity and were judged by the Investigators to be clinically insignificant . The abnormal tests were the red blood cells, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV). Based on all of the results obtained in this clinical study, it is concluded that the test food supplement given in the tested doses have no safety problems of clinical significance. The other investigations in humans were at lower dosages. The Study on the Safety of Oral ALA Phosphate (Alone and in Combination with Sodium Ferrous Citrate) in Healthy Adults, Protocol No.: 19474 June 17, 2008 was submitted by Cosmo Oil Co. Ltd. at the Meguro Medical Clinic, Medical Corporation Yukokai.[10] The Investigator is Hitoshi Suzuki, MD. There were 30 participants that consumed 97.4% of the additive. The food supplement additive was taken for 4 weeks with a 2 week follow-up. The investigators report no related symptoms and document some statistically significant laboratory changes, but not of clinical significance. The following summarizes these findings.

Laboratory Examination Parameters and their mean values showed statistically significant P=<0.05 changes from baseline at indicated time points from intervention group:

Parameter	**Percent increase or decrease**	
Red blood cells (x104/μL)	-2.5%	(week 4)
Hemoglobin (g/dL)	-3.3%	(week 4)
	-2.8%	(post week 2)
Hematocrit	-3.7%	(week 2)
	-4.2%	(week 4)
MCV (fL)	-1.7%	(week 4)
MCH (pg)	-1.0%	(week 2)
HDL (mg/dl)	-5.7%	(week 4)
Urinary pH	-9.0%	(week 2)

Many parameters resolved over time and the clinical implications of these findings were judged to be without clinical consequence.[10]

Another investigation conducted to study anemia involved 104 female subjects, ages 20-65 with Hemoglobin levels 8-12 g/dl over a 12 week period. Daily doses of food supplementation were administered and expressed as 5-ALA phosphate / sodium ferrous citrate: 10.0 mg / 92.0 mg (high-dose), 5.0 mg / 46.0 mg (mid-dose), and 2.5 mg / 23.0 mg (low-dose).[2, 11, 12] This investigation also resulted in no safety problems of clinical significance. Another very recent investigation on 6 subjects with high levels of HbA1C was also conducted (Tables 6 and 7). These data suggested a decline in HbA1C. Lastly, another study conducted at the University of Hawaii, John A. Burns School of Medicine, The Pre-Diabetes and Supplement Study, involved 154 participants divided into three groups. Daily doses of 5-ALA phosphate / sodium ferrous citrate were: 15.0 mg / 17.2 mg (low-dose), 50 mg / 57.4 mg (high-dose) and control, over a

12 week period. In 2 hour post-Oral Glucose Tolerance Tests (OGTT), glucose levels declined significantly compared to those not taking the supplement ($p=0.02$).[7] HbA1C results were of borderline significance ($p=0.07$) and no untoward effects were reported according to Rodriguez BL, Curb JD, Davis J, et al.[7] Again, all of these studies had no safety problems of clinical significance.[7, 13, 14]

None of the investigations document the presence of symptoms or abnormal laboratory results of clinical significance. Minor laboratory changes were judged to be not clinically significant. For the following investigation, diarrhea and abdominal pain were incorporated into the informed consent and the protocol was modified to add laboratory assessments at 4 and 8 weeks into the study. The University of Hawaii Institutional Review Board approved the investigation.

Sleep and 5-ALA

In a previous study conducted to examine the relationship between 5-ALA and pre-diabetes, a questionnaire covering a wide range of measures of health was administered. A daily diary was also completed by the participants. The study placed participants on the 5-ALA supplement for a period of 12 Weeks. The participants were surveyed with the questionnaire at Week 0 and Week 12, and were also interviewed, and the daily diary reviewed, at Week 0, 4, 8, 12, and 16. During the course of this study, some interesting results relating to sleep patterns emerged. Table 3 provides a summary of the survey results pertaining to sleep in some of the participants.

These data support the hypothesis that 5-ALA may be related to improved sleep. The fact that sleep patterns improved while on the 50 mg supplement, and then returned to previous patterns when the supplement was stopped, provide strong rationale for this pilot investigation.

Hypothesis Related to Sleep

There are several possible mechanisms hypothesized for the improvement in sleep, because 5-ALA may have an impact on increasing the energy of all cells. In one study involving test mice, researchers found that the regular administration of 5-ALA appeared to raise serotonin levels in the brain.[15] This may explain the improvements in sleep patterns.

Another possible hypothesis is that 5-ALA helps each cell's metabolism, such that its own circadian rhythms are better defined. One may also speculate that 5-ALA may support hormonal regulation, including melatonin production, in the pineal gland - which may result in better sleep and corticosteroid production in the adrenal glands. This may also assist in dealing with stress.

CHAPTER 2. METHODS

Design

This was a double-blind, randomized parallel-group comparison study.

Sample

40 participants were randomized to the following 2 study groups: Control Group - 20 participants, and Intervention Group - 20 participants. A table of randomized numbers was used to assign the participants.

Procedures

Recruitment. Advertisements were prepared for the newspaper, TV and radio. Postcards were mailed. Flyers and newspaper ads were distributed and local MD's and organizations were contacted. Drs. Shintani and Rodriguez gave seminars and spoke at community events.

Participants in the intervention group took one 50 mg capsule of 5-ALA each day, orally. Controls were provided with a placebo of similar size and color.

Inclusion Criteria. Both males and females were equally recruited. Participants were given questionnaires, and those who were between 40 and 70 years who self-reported having insomnia or difficulty sleeping were selected.

Exclusion Criteria. Those at a body weight of <110 or >250 lbs were excluded. Those who were taking any supplements or medications for sleep were also excluded. Those with a history of porphyria were not able to participate, as 5-ALA may cause adverse effects on porphyria patients as it effects porphyrin

metabolism. Those with a history of hemochromatosis were excluded, as sodium ferrous citrate (SFC) may cause adverse effects on hemochromatosis patients which have defects in iron metabolism. Those with a history of hepatitis were also excluded, as SFC may cause an allergic reaction in this population. Those with active liver disease and iron sensitivity were also excluded. Women who were pregnant, breastfeeding, and those participating in another clinical study were excluded. Those with ferritin levels elevated above 125% of normal on screening were excluded.

Assessments

Sleep Study Questionnaire

Lifestyle-related Questionnaire

Medical history, occurrence of allergy, use of medical drugs and / or health food, drinking, smoking habits, etc.

Clinical Examinations

Body weight, BMI, Seated/Resting Systolic and Diastolic Blood Pressure, Waist Circumference

Laboratory Tests: CBC, Ferritin

Venous blood was obtained and all analyses were conducted using standardized and certified procedures by Diagnostic Laboratory Services (DLS). Samples for selected tests were stored by DLS at appropriate temperatures for assay testing.

Other Measurements

The same digital scale was used for weighing patients at each site throughout the study. Height was measured in the standing position and measured to within the nearest millimeter without shoes. A stadiometer fitted with a vertical backboard, fixed floorboard, and a movable headboard was utilized. BMI was determined as weight (kg) divided by height squared (m^2). Waist circumference was measured using a tension-controlled measuring tape while the subject is standing. Abdominal obesity is defined as a WC ≥88 cm in women and ≥102 cm in men. Blood pressure was measured with a digital sphygmomanometer while patients are in a sitting position. The same arm was used to obtain all readings over the course of the study, unless contraindicated. Sitting systolic and diastolic blood pressures were estimated by averaging 2 replicate measurements obtained 1 to 2 minutes apart. When possible, the same nurse or study coordinator measured waist circumference at each visit to ensure consistency. Leisure-time physical activity and general health was measured using a Lifestyle Questionnaire. Sleep was measured using a SSS Survey and the *Pittsburgh Insomnia Rating Scale – 20 Questions (PIRS-20),* along with a daily study diary to monitor consistent sleeping or mood patterns. The PIRS-20 is copyrighted by the University of Pittsburgh.[16] Items must occur in their original sequence, as this aspect is a deliberate design feature. The PIRS-20 has repeated measures of validity. The PIRS-20 is available as an open resource. A lower score indicates better sleep: Scale - 0 (good) to 60 (bad).

Results from the lab were reviewed by the medical staff / physician within three business days of receiving the results. There was follow-up activity by contacting the participant and the participant's physician, as appropriate.

Study Schedule

Participants took the supplement from enrollment. The measurements occurred at baseline, week 3, week 6 and week 10. At week 10, participants were no longer taking the supplement (see Table 5).

Efficacy Assessments

A significant difference between sleep and related measures of sleep quality during the examination period between the treatment groups was the major outcome measure.

A significant difference in sleep scores between the treatment group and control group at the end of administration is desired and reflective of affirming the hypothesis.

Stopping criteria as related to signs and symptoms and referrals for care

The stopping criteria as it relates to possible adverse events, such as participant's complaints and physical and laboratory abnormalities were as follows:

If participants develop symptoms that are persistent and / or intolerable (examples: diarrhea, abdominal pain / discomfort, or flatulence), participants

would be asked to stop the study capsules. Any abnormalities in blood tests would make participants ineligible for the study, or if enrolled, would require stopping the study capsules. In addition, if participants have a major illness, hospitalization or surgery during the study period, they were asked to stop the study capsules. All abnormal findings were documented in the study chart and data entered, so that data on adverse events can be analyzed. Data on adverse events were analyzed every two weeks.

All abnormal findings, including reported symptoms, laboratory results or abnormal findings in the physical examination would be reviewed by the study nurse and / or Dr. Shintani / Clinical Director to insure appropriate follow-up. He would contact the participant's primary care provider by phone if the findings required urgent attention. Participant permission to contact the primary care physician was requested at the time of enrollment. If participant had given written consent, primary care providers would receive a copy of the laboratory tests and were given contact information for the clinic, in case the physician has questions related to the study. The results were mailed to the physicians within one week of receiving the final results from the lab at the study clinic.

Examples of urgent referrals not related to the study that may be encountered and that would require immediate follow-up are: blood pressure greater than 210mmHg systolic or 105mmHg diastolic or resting pulse rate <40/min or >130/min. The participant would also be advised of these abnormal findings and be asked to follow-up with their physician, as appropriate.

Interim Analyses and Stopping Trial Early (Data Safety Monitoring)

Poor recruitment, new evidence, harm, superiority or futility was to all be considered grounds for terminating the trial early. Data was examined continuously during recruitment and analyses. This would have been used as the means of assessing the adequacy of the design and determining if the trial should be stopped early.

Adverse events data were assessed every two weeks to identify statistically or clinically significant differences between the control group and intervention group, and assessments of whether or not changes to the protocol were needed. If statistically or clinically significant differences in serious adverse events among the placebo and the diet supplement group were found that indicate that there is greater risk than benefit for study participants, the study could have ended. An experienced statistician, Dr. James Davis has been assisting Mr. Michael Perez with conducting the statistical analyses.

Participants were advised to contact the PI or Co-PI immediately if they have any concerns. Any abnormal findings were reviewed, reported and the participant would have been removed from the investigation, if warranted.

Arrangements were made for packaging the supplement at the factory in Japan. The supplements were placed in white plastic bottles. Each bottle contained a 45-day supply. Shipping, insurance and import requirements have been established with the manufacturer.

Statistical Analysis

Variables monitored as part of the evaluation were assessed by comparing the intervention group to the control group. Two-sample t-tests were used to assess statistical significance at baseline and follow-up exams between the control and intervention groups. Baseline data were summarized as means and standard deviations with differences among the randomized groups tested for significance by t-tests and chi-square tests. To measure the possible differences in rates of change in sleep and mood scores across follow-up time between the 5-ALA treatment and the control group, an additional analysis was implemented consisting of an estimate of differences in slopes using a linear regression model. Mixed linear models were fit using the proc mixed procedure in SAS 9.2.[17] The regression models included an indicator variable identifying treatment groups, a variable for weeks of follow-up, and interaction terms between the indicator variables and follow-up time. Results were summarized as the difference in slopes comparing the intervention groups to the control group. Results were also presented graphically to illustrate the estimated differences in slopes for the study groups. All significant tests were two-sided. Differences will be considered significant if the p values are ≤ 0.05.

CHAPTER 3. RESULTS

Of the 40 participants in the Sleep study, refer to Table 8, the mean age for the control group was 54.7 years and for the intervention group 56.3 years. Seventy percent of the participants were female in the control group opposed to 65% female in the intervention group. The analysis of variance (ANOVA) was used to test statistical significance for age by supplement, the p value was .494 and it is not significant. A Fishers exact test was used to determine statistical significance of race by supplement. The p value was .905 and it is not significant. A Fishers exact test was used to determine statistical significance of gender by supplement. The p value was 1 and it is not significant. No significant differences were identified between the groups related to the demographic characteristics.

The PIRS-20 scale shows sleep improvement when the score is lowered. The control group (see Figure 2 and Table 9) and the slight change in sleep improvement could be attributed as a random result from baseline to week 3. As week 3 to week 6 show no response in either direction. On week 10, when no longer taking the supplement, there was no change. The 5-ALA 50 mg group (see Figure 3 and Table 10), the PIRS-20 score declined from baseline to week 3 and again on week 6. Week 10, when no longer taking the supplement, the response begins to return to baseline. This is the expected outcome.

Improvement in sleep in the group taking 50 mg 5-ALA, compared to controls, was significant. The mean change, from Baseline through week 6, was -5.67 units less on the sleep scale than the control group with a p value of .001. The

mean change from week 6 to week 10 when the participant was no longer taking the supplement was 4.55 units higher than the control with a p value of .062, which is of borderline significance.

In addition, No clinically significant abnormalities were observed in the sleep study or any of the previous studies, that could be attributed to the food supplement.

CHAPTER 4. DISCUSSION

Hypothesis

Insomnia prevalence in the general population is estimated at 30-50% .[18] Medications currently used to treat sleep disorders like diphenhydramine, doxylamine and antihistamines have negative side effects. Diphenhydramine side effects include: dry mouth, dizziness, prolonged drowsiness lasting into the next day and memory problems.[19] Doxylamine side effects include: asthma, bronchitis, glaucoma and peptic ulcer or enlarged prostate.[20] Antihistamines can cause dry mouth, urine retention and blurred vision. In addition, all sleep medications cause drowsiness as a solution for treating sleep disorders. Remarkable improvement in sleep was reported by several participants in a previously conducted study investigating the relationship between the dietary supplement 5-ALA and pre-diabetes. The dietary supplement 5-ALA suggested being a potential alternative approach to improved sleep. 5-ALA creates energy and may adjust a person's circadian cycle in order to allow for better sleep in a natural way. For this reason there is a great interest on natural alternatives for the treatment of sleep disorders.

Increased level of glucose in the cell results in decreased 5-ALA production. Decreased heme production occurs during aging, and at age 40 the human body produces approximately 50 mg/day less.[7] This results in decreased hemoglobin production with decreased heme enzyme activity. A decline in the mitochondrial

electron transfer system (the primary cellular energy producer) follows, with decreased basal metabolism - as well as physical energy decline.[7]

Melatonin plays a role in regulating circadian rhythms and maintaining physical energy. Melatonin is a chemical that occurs naturally in the brain. Melatonin allows a person to become sleepy. Melatonin is naturally produced during the day-shift phase, or phase-delay (see Figure 4).

Endogenous melatonin is produced by the human body. Production begins about two hours before bedtime, provided that the lighting is dim. “This is known as dim-light melatonin onset, DLMO.”[21] What results is the phase-advance portion of the phase response curve (PRC) (Figure 4). This assists with regulation of the sleep-wake schedule.[22]

According to R.L. Sack, et al, circadian rhythm sleep disorders all involve a problem in the timing of when a person is asleep and is awake.[23] He proposes a master circadian clock in the brain called the suprachiasmatic nucleus (SCN).[24] The SCN controls the timing of body rhythms related to temperature and hormone levels over a cycle that lasts a little longer than 24 hours.

In order for this system to function efficiently, it needs information from a variety of sources, which include physical activity, social activities and to experience day and night. The ganglion cells in the retina collect light information for the SCN. These cells produce a pigment called melanopsin and are particularly sensitive to light.[25] This is true for “non-image visual functions, such as circadian photo-entrainment and the pupillary light reflex”.[25] The major conduit for rod and

cone signals to the brain for non-image visual functions, such as circadian photo-entrainment and the pupillary light reflex.[25] Light exposure is needed by the pineal gland to produce melatonin and the day-phase contributes to melatonin production (see Figure 4).[26] Melatonin allows a person to be sleepy. "Melatonin is a hormone produced by the pineal gland that contributes to the reinforcement of circadian and seasonal rhythms."[27] It also helps prepare the body for sleep when melatonin enters phase-advance (see Figure 4) and light reduction enters the phase-advance cycle. Extended or enhanced day-phase activity could result in increased melatonin during the evening. 5-ALA could, in fact, enhance day-phase activity, which could inversely allow a person to experience a better sleep cycle.

This is potentially very important because it may make for an ideal sleep-aid that assists in inducing drowsiness at night and wakefulness during the day and minimizes the unwanted side effects related to drowsiness. It should be noted that this particular research was not designed to confirm any change in melatonin levels in the pineal gland. The methods and design did not include testing for melatonin production. Studies to evaluate the hypothesis regarding the clinical significance of the use of 5-ALA and a relationship with sleep are needed.

Conclusions and Recommendations

There appears to be a relationship between the administration of dietary supplements containing 5-ALA and sleep. The results of this study suggest that 5-ALA does, in fact, improve sleep. The mechanism for sleep improvement

needs to be explored. Further research is warranted to explore the mechanism by which 5-ALA may play a role in the improvement of sleep.

Current research about 5-ALA and sleep is that 5-ALA has an indirect relationship with intra-cellular energy production and an effect on potentially neuroactive substances such as tryptophan, serotonin, or melatonin. Enhanced cellular energy production could result in a wide range of effects from cellular to endocrine to neurologic. 5-ALA as a key component of heme and the cytochrome system appears to have an impact on increasing the energy of all cells. Research on cytochrome C oxidase activity and ATP levels in mice in response to 5-ALA appear to confirm this effect.[13] In addition, animal studies indicate a possible effect on neurotransmitters which may have an effect on the sleep-wake cycle.

Many sleep medications induce a tolerance and are recommended only for short-term use. 5-ALA may assist in the adjustment of a person's circadian cycle, endocrine function or neurologic function in order to allow for better sleep in a natural way. In doing so it may provide for a safer alternative to currently available sleep medication. Further research is needed to explore this possibility.

Table 1

*** Common Foods That Contain 5-ALA**

FOOD	*5-ALA content*	
Spinach	**0.18**	**mg/kg**
Green pepper	**0.23**	**mg/kg**
Tomato	**0.13**	**mg/kg**
Shitake mushroom	**0.60**	**mg/kg**
Potato	**0.12**	**mg/kg**
Banana	**0.40**	**mg/kg**
Squid	**0.50**	**mg/kg**
Octopus	**1.00**	**mg/kg**

FERMENTED PRODUCTS		
Shochu lees	**70**	**mg/kg**
Sake lees	**9-26**	**mg/kg**
Baker's yeast	***140***	***mg/kg***
Wine	***5-ALA content*** **1.4-2.2**	***5-ALA mg/L*** **mg/L**
Vinegar	**0.1-5**	**mg/L**
Sweet sake	**0.4-6**	**mg/L**
Sake for cooking	**0.3-13**	**mg/L**
Sake	**0.9-4.5**	**mg/L**
Soy sauce	**0.3**	**mg/L**

*** As can be seen from a review of the above table, 5-ALA is found in many common foods.**

Table 2

Summary of Toxicity Studies of 5-ALA Phosphates

Preclinical and Safety Tests	**Dose or Response**	
Acute oral toxicity	**LD 50 > 200 mg/kg**	**Good Laboratory Practice (GLP)**
Subacute oral toxicity (28 days)	**60 mg/kg/day**	**GLP**
Chronic oral toxicity (90 days)	**15 mg/kg/day**	**GLP**
Reversion test with bacteria	**negative**	**GLP**
Chromosomal aberration test with mammalian cells in culture	**negative**	**GLP**
Reproduction and development toxicity administration of the drug prior to, and in early stages of, pregnancy	**60 mg/kg/day**	**GLP**
Reproduction and development toxicity administration of the drug during the period of fetal organogenesis	**180 mg/kg/day**	**GLP**
Reproduction and development toxicity administration of the drug during the perinatal and lactations periods	**180 mg/kg/day**	**GLP**

Acute dermal irritation	**negative**	**GLP**
Continuous dermal irritation	**negative, 6 (w/v) %**	**GLP**
Conjunctival irritation	**negative**	**GLP**
Skin sensitization	**negative**	**GLP**
Skin photosensitization	**negative**	**GLP**
Phototoxicity	**negative, 6.0 (w/v) %**	**GLP**
Patch test	**negative, 0.6 (w/v) %**	**non-GLP**

The Table provides a summary of the toxicity studies done using 5-ALA. Significant safety is demonstrated. [2, 28]

Table 3

Results of Pilot Investigation Related to Sleep

	Sleep at Week 0 (first day to start 5-ALA)	Sleep at Week 12 (12 wks 5-ALA intake)	Sleep at Week 16 (4 wks post 5-ALA)
Case # 4	6.5 hours, wakes up several times	7.25 hours, mostly continuous sleep	Pattern reverting to waking up several times at night & less restful sleep
Case # 138	8.0 hours sleep and does not take a "nap" during the day, "Moderate" energy	7.5 hours, able to take a 10 min. "nap" during the day, now reports having "Normal" energy	Feels "tired", "no energy", "sleepy"

Table 4

PROTOCOL FOR SLEEP STUDY

	Control	**Intervention**
5-ALA Phosphate	0 mg	50 mg
Sodium Ferrous Citrate (SFC)	0 mg	57.4 mg (6.08 mg as Fe)
Others	Alpha starch, Silicon dioxide	Alpha starch, Silicon dioxide

Table 5

Study Schedule

Study Schedule	**Screening**	**Intervention Period**	**Intervention Period**	**Intervention Period**	**Follow-up**
VISIT SCHEDULE	**Screening**	**Week 0** **(Visit 1)** **Baseline**	**Week 3** **(Visit 2)**	**Week 6** **(Visit 3)**	**Week 10** **(Visit 4)**
Lifestyle Questionnaire Sleep-related Questionnaire SSS Questionnaire		●	●	●	●
Clinical Examinations **Including physiological Measurements**	●	●	●	●	●
Laboratory Tests: CBC and Ferritin	●				

Study Schedule	Screening	Intervention Period	Intervention Period	Intervention Period	Follow-up
Level					
Intake of Study Pills		●	●	●	
Filling of Study Diary		●	●	●	●
Review Participant Concerns	●	●	●	●	●

Table 6

Studies Completed or In Progress

(Cosmo Oil and SBI ALApromo)

Type of study	Species	Method of Administration	Duration of Dosing	Doses	Period of Study
A 13-Week Oral Dose Toxicity Study of δ-Aminolevulinic acid Phosphate with Sodium Ferrous Citrate (SFC) in Rats	Rat	p.o.	91 days	with SFC (1:0.5) 50, 125, 250 mg/kg/day; without SFC 50,125 mg/kg/day	June 09 -Sep 09
A 4-Week Oral Dose Safety Study of δ-Aminolevulinic acid Phosphate with Sodium Ferrous Citrate in Human	Human	p.o.	28 days	25, 50, 75, (100) mg/body/day ALA:SFC = 1:0.5	June 09 - Feb 10
Study on Antidiabetic Effect of Oral δ-Aminolevulinic acid (ALA) Phosphate in Genetically Type 2 Diabetic Rat (ZDF rat)	Rat	p.o.	28 and 56 days	2 mg/kg/day with 2.3 mg/kg/ day SFC	July 09 - Oct 09
Evaluation of the effect of δ-Aminolevulinic acid phosphate and sodium ferrous citrate on basal metabolism of rats	Rat	p.o.	TBD	TBD	TBD
Evaluation of the beneficial effect of supplementary diet containing δ-Aminolevulinic acid phosphate and sodium ferrous citrate on adults with marginal diabetes (Proof of Concept Study)	Human	p.o.	TBD	TBD	TBD

Table 7 **Finished Toxicity & Efficacy Studies** **(Cosmo Oil & SBI ALApromo)**

Type of study	Species	Method of Administration	Duration of Dosing	Doses	GLP Compliance	Results
Single-Dose Toxicity						
Single-dose Oral Toxicity Study δ-Aminolevulinic acid Phosphate in Rats	Rat	p.o	single dose	2000mg/kg	Yes	Ld50 > 2,000 mg/kg
Repeat-Dose Toxicity						
A 4-Week Oral Dose Toxicity Study of δ-Aminolevulinic acid Phosphate in Rats	Rat	p.o	28 days	60, 250, 500 and 1000 mg/kg/day	Yes	NOAEL 60mg/kg/day
A 4-Week Oral Dose Toxicity Study of δ-Aminolevulinic acid Phosphate in Rats with a 4-Week Recovery Period	Rat	p.o.	90 days	15, 60 and 250 mg/kg/day	Yes	NOAEL 60mg/kg/day
Analysis of Blood Porphyrin analogs Concentration in a 13-Week Oral Dose Toxicity Study of δ-Aminolevulinic acid Phosphate in Rats	Rat	p.o.	90 days	15, 60 and 250 mg/kg/day	No	
Analysis of Blood PPIX Concentration in a 13-Week Oral Dose Toxicity Study of δ-Aminolevulinic acid Phosphate in Rats	Rat	p.o.	90 days	15, 60 and 250 mg/kg/day	No	

Study on the Safety of Oral ALA Phosphate (Alone and in Combination with Sodium Ferrous Citrate) in Human Healthy Adults	Human	p.o	28 days	5, 15 mg/body/day ALA only 5 mg/body/day ALA + 2.87 mg/body/day SFC	No	Safe
Anrigenicity and Skin Sensitization						
Skin Sensitization Study with δ-Aminolevulinic acid Phosphate in Guinea Pigs (Maximized method)	Guinea Pig	Topical	24 or 48 hours	Sensitization 50 w/v % Challenge 30, 10, or 1 w/v %	Yes	Positive reactions noted at each concen-tration only in the sensiti-zation induction group.
Skin Sensitization Study with δ-Aminolevulinic acid Phosphate in Guinea Pigs (Buehler method)	Guinea Pig	Topical	6 hours	Sensitization 50 w/v% Challenge 30, 10, and 1 w/v%	Yes	Negative
Photosensitization Study with δ-Aminolevulinic acid Phosphate in Guinea Pigs (Adjuvant and Strip-AA method)	Guinea Pig	Topical + Light Irradiation		Sensitization 60 w/v% Challenge 50, 10, and 1 w/v%	Yes	Positive at both irradiated and non-irradiated sites only in the sensiti-zation induction group.

Photosensitization Study with δ-Aminolevulinic acid Phosphate in Guinea Pigs (Harber Method)	Guinea Pig	Topical + Light Irradiation		Sensitization 60 w/v% Challenge 50, 10, and 1 w/v% - UV-B 1 J/ cm2 then UV-A30	Yes	Negative
Local Tolerance						
Primary Skin Irritation Study with δ-Aminolevulinic acid Phosphate in Rabbits	Rabbit	Topical	24 Hours	6 w/v% solution	Yes	Negative
Fourteen-day Skin Irritation Study with δ-Aminolevulinic acid Phosphate in Rabbits	Rabbits	Topical	14 Days	6 w/v% solution	Yes	Negative
Eye Irritation Study with δ-Aminolevulinic acid in Rabbits	Rabbit		Single Dose	6 w/v%	Yes	Considered to be minimally irritating
Phototoxicity						
Photo Irritation Study with δ-Aminolevulinic acid Phosphate in Guinea Pigs	Guinea Pig	Topical + Light Irradiation	Single Dose	6 w/v% - 0.6 j/cm2, 9 j/cm2	Yes	Negative
Human Patch Test						
Human Patch Test with 0.6 w/v% Solution of δ-Aminolevulinic acid Phosphate	Human	Patch	24 Hours	0.6 w/v%	No	Negative
Study on Antidiabetic Effect of Oral δ-Aminolevulinic acid (ALA) Phosphate in Genetically Type II Diabetic Mice (KK-A^y Mice)	Mouse (KK-A^y)	p.o.	28 Days	10 + 92, 30 + 276 mg/kg/day (ALA + SFC)	No	Dose dependent prevention of increase of blood sugar level

Evaluation of the beneficial effect of supplementary diet containing δ-Aminolevulinic acid Phosphate and Sodium Ferrous Citrate on blood hemoglobin level in adult females with mild anemia (DOSE-finding study)	Human	p.o.	74 Days	0 + 46, 2.5 + 23, 5 + 46, 10 + 92 mg/body/day (ALA + SFC)	No	Dose dependent effect on anemia - No significant adverse effect

Table 8

Descriptive Statistics

N=40	Control		50 mg	
	Mean	SD	Mean	SD
Age	54.75	7.91	56.3	6.17

50 mg N=20	%	Mean Age
Asian / Filipino	50%	57.9
Native Hawaiian	20%	53
Caucasian	25%	58.8
Hispanic / Latino	0%	
African American	5%	63
American Indian / Alaska Native	0%	
Female	70%	54.9
Male	30%	59.5

Control N=20	%	Mean Age
Asian / Filipino	45%	53.8
Native Hawaiian	10%	62
Caucasian	30%	56.16
Hispanic / Latino	5%	43
African American	5%	53
American Indian / Alaska Native	5%	53
Female	65%	54.61
Male	35%	55

Figure 1

5-Aminolevulinic Acid

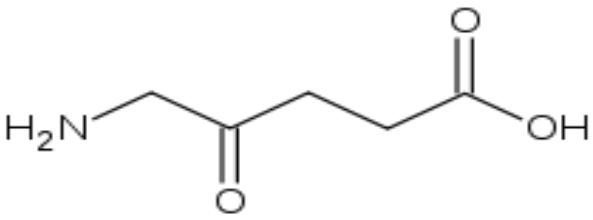


Figure 2

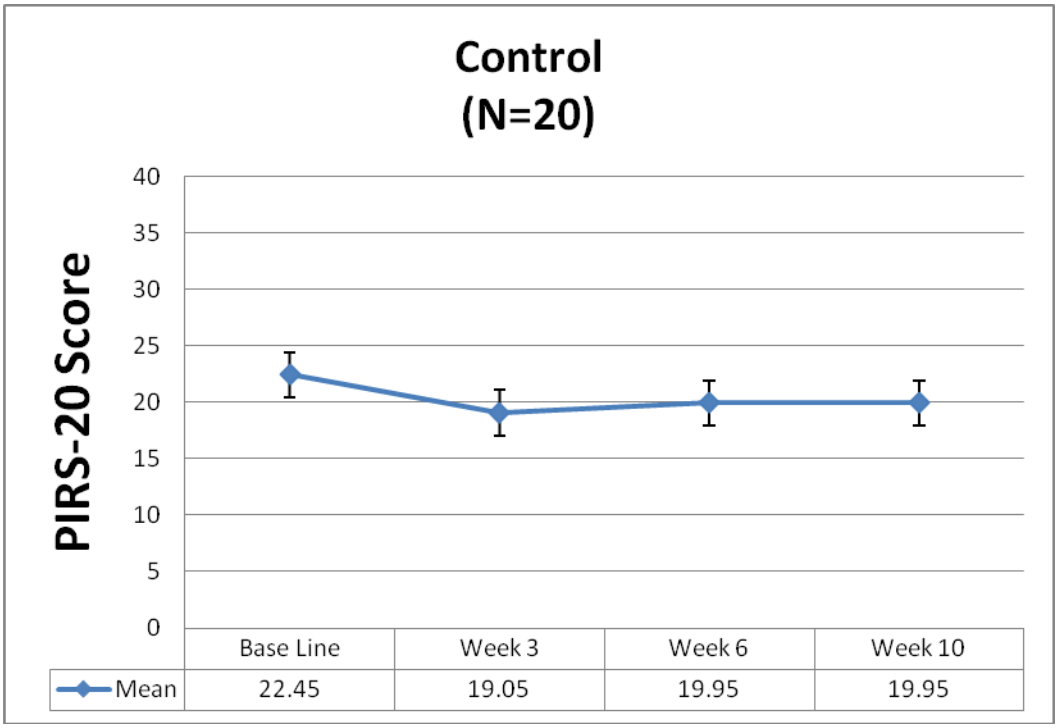


Table 9

Control	Mean
Base Line	22.45
Week 3	19.05
Week 6	19.95
Week 10	19.95

Figure 3

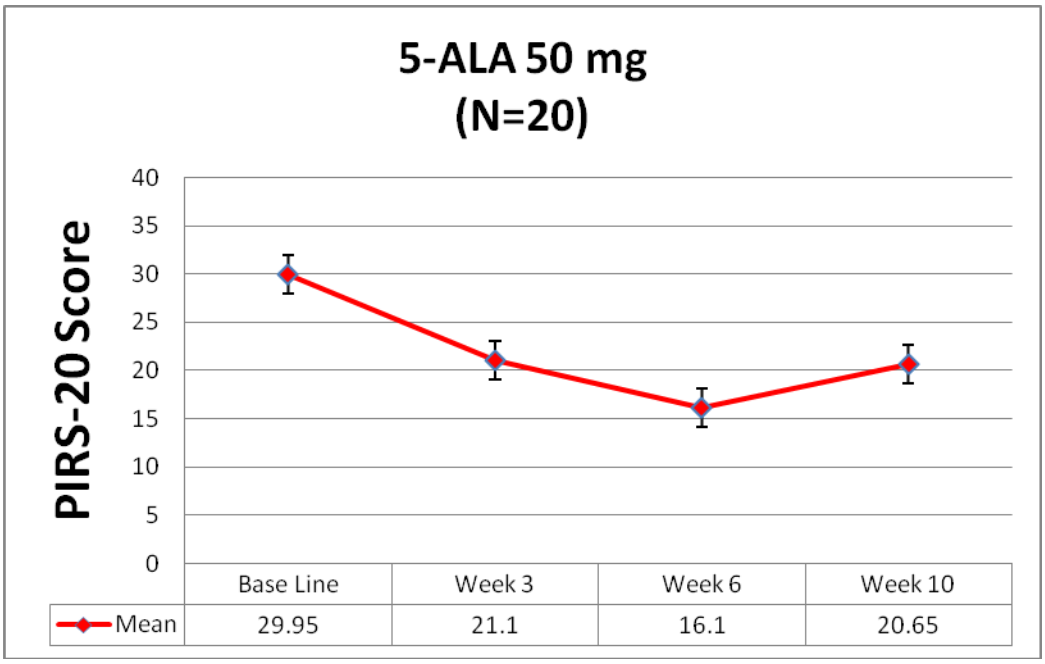
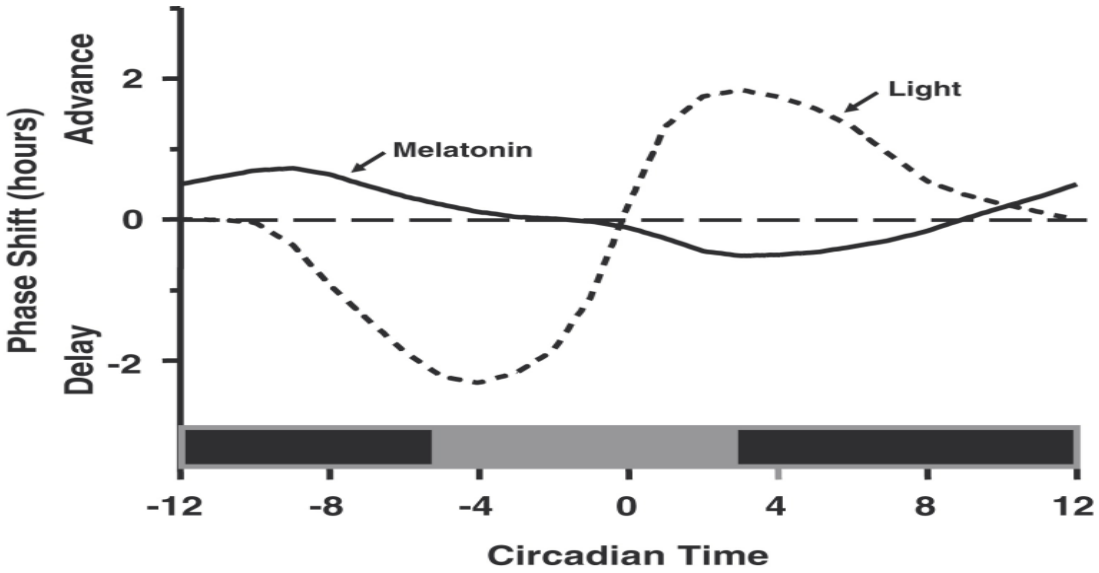


Table 10

50 mg	Mean
Base Line	29.95
Week 3	21.1
Week 6	16.1
Week 10	20.65

Figure 4

Phase Response Curve (PRC)



Adapted from Lewy et al. and Khalsa et al. with permission.

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