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Brown seaweeds are popular foods in Japan, where the incidence of breast cancer is about 1/6 the rate of that reported for American women. Seaweed is an excellent source of fiber, contains iodine, carotenoids, and both mammalian lignans and isoflavones. Seaweed may help to prevent breast cancer by several different mechanisms involving these and other constituents. In a preliminary study of toxicity and efficacy using 5 g/day of seaweed, we found seaweed was well tolerated and was associated with some biological changes in the variables measured. In this study, we will examine the effects of escalating doses of seaweed supplementation in a group of healthy postmenopausal women with and without breast cancer. In our cross-over design, women will be randomized to either seaweed or placebo first. We will then give doses of seaweed or placebo (3 g/day for 3 weeks, then 6 g/day for 3 weeks), followed by 1 week of 6 g/day seaweed/placebo plus soy (2 mg isoflavone per kg bodyweight). Our primary outcome variables are changes in circulating estrogen levels, thyroid hormones, and urinary excretion of phytoestrogens. Adherence to our program will be measured by monitoring urinary excretion of iodine.

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Jane Tras — June 7, 1999

PI - Signature — Date

Table of Contents

Dietary Seaweed and Early Breast Cancer: a Randomized Trial

Introduction

Brown seaweeds are popular foods in Japan, where the incidence of breast cancer is about 1/6 the rate of that reported for American women. Seaweed is an excellent source of fiber, contains iodine, carotenoids, and both mammalian lignans and isoflavones. Seaweed may help to prevent breast cancer by several different mechanisms involving these and other constituents. In a prelimary study of toxicity and efficacy using 5 g/day of seaweed, we found seaweed was well tolerated and was associated with some biological changes in the variables measured. In this study, we will examine the effects of escalating doses of seaweed supplementation in a group of healthy postmenopausal women with and without breast cancer. In our cross-over design, women will be randomized to either seaweed or placebo first. We will then give doses of seaweed or placebo (3 g/day for 3 weeks, then 6 g/day for 3 weeks), followed by 1 week of 6 g/day seaweed/placebo plus soy (2 mg isoflavone per kg bodyweight). Our primary outcome variables are changes in circulating estrogen levels, thyroid hormones, and urinary excretion of phytoestrogens. Adherence to our program will be measured by monitoring urinary excretion of iodine.

Body

Task 1. Develop Plan for Study Computer Database, Months 1-3

a. **Normal study values will be entered for each outcome variable, so out-of range values will immediately alert investigators to potential problems.**
   Since all analyses are being performed at the end of the study, rather than concurrent with the study, and normal values may not be relevant, we are plotting the values longitudinally for each patient to see where an individual's values might have varied.

b. **Tracking system will be developed to monitor each volunteer, and to record data from laboratory analyses, medical histories, interviews and diaries.**
   The tracking system has been developed and is being used.

c. **Train project coordinator in patient-centered counseling to be used in this study.**
   Project coordinator was trained in patient-centered counseling.

d. **Orient the staff to the study, all of whom work in the Division of Preventive and Behavioral Medicine.**
   Staff was oriented to the study, and understood the overall purpose and how it was to be run.

Task 2. Seaweed, Months 1-3

a. **Identify exact location of seaweed to be used, visiting the collection site, overseeing drying, grinding, and encapsulation processes.**
   Three seaweed harvesters were identified, and each was visited (1 site in British Columbia, Canada; 1 site in the San Juan Islands (WA); and 2 sites in Maine) to evaluate harvesting techniques, reliability of location identification, age of plants harvested, transportation methods, drying methods, and grinding facilities. Two harvesters (Larch Hansen of Maine Seaweed Company, located in Stuben, Maine) and Ryan Drum of Island Herbs, located on Waldron Island, Washington) were chosen, and seaweed ordered.
   About 10 encapsulators were interviewed. Of these, two were chosen for possible use. Beehive Botanicals was chosen as the encapsulator of choice based on a site visit to their plant, located in Hayward Wisconsin. That facility was found to adhere to good practices of quality control and hygiene and the method of encapsulating was highly reliable.

b. **Overseeing grinding and encapsulation of oatmeal for the control supplement.**
   Because of its mucopolysaccharide properties, oatmeal was replaced by Maltodextrin, a more biochemically neutral placebo. The Maltodextrin was encapsulated by Beehive Botanicals, under the same conditions as the seaweed encapsulation. Both the seaweed and the placebo capsules are made of white gelatin. The capsules were analyzed for iodine content, and found to have none, and samples of the final seaweed capsules were

analyzed for iodine content, and found to be approximately 100 mcg/g for the Alaria used in the pilot study.

c. Analysis of seaweed for iodine and seaweed and oatmeal for percentage of soluble and insoluble fiber.

We decided to rely on existing fiber content analysis done by Maine Coast Sea Vegetables on the Alaria, and the company analysis of Maltodextrin. In the second part of the study, using two different seaweeds, we will analyze each for fiber content.

Task 3. Pilot Test, (proposed for Month 4) actually completed by month 7.

a. Pilot test and refine data collection instruments.

1. Twenty-two commercially available seaweeds were analyzed for iodine content. The iodine content varied from 30 mcg/g to over 8,000 mcg/g. Since iodine supplementation of more than 1,000 mcg/d was considered by Dr. Lewis Braverman of Harvard Medical School (and collaborator on this study) to be potentially toxic, the original choice of Laminaria was changed to two other commonly eaten brown seaweeds, Sargassum and Alaria. The iodine content of Laminaria ranged from 2,000 mcg/g to over 8,000 mcg/g. Our target dose is 5 grams/day. The iodine content of Sargassum is 30 mcg/g, (we would be providing 150 mcg/d in our study), and the iodine content of Alaria is 100 mcg/g (we would be providing 500 mcg/d in our study). There is some evidence that supplementary iodine may be helpful in preventing fibrocystic breast disease, and possibly breast cancer, so we chose Alaria for our initial pilot study, and will use Sargassum and Alaria in the escalating dose study.
2. We did a priliminary study of 2 volunteers who took seaweed capsules with and without soy powder, to see if seaweed was likely to influence urinary phytoestrogen excretion. We found that seaweed alone made only minor differences, but seaweed plus soy made a 100-fold difference in the excretion of equol. Equol is thought to be the phytoestrogen of particular importance in breast cancer, and only about 1/3 to ½ of all Americans is an equol producer. The synergism of seaweed (a fiber source) and soy seemed to make one of the volunteers become an equol producer. The other woman was already an equol producer, and the addition of seaweed made no difference in her rate of equol excretion.
3. Based on this finding, we modified the pilot study to include 6 weeks of seaweed/placebo followed by a week of seaweed/placebo plus soy. We wrote a small grant to Protein Technologies and obtained high isoflavone soy powder for use in the study.
4. In order to account for important food sources of phytoestrogens, questions on high phytoestrogen-containing vegetables were added to The Seven Day Dietary Recall Questionnaire.
5. Health diaries were designed for use by the participants to record vegetables, alcohol, and any medication changes that occurred.

6. Fabric bags were designed and sewn, for carrying the 6 liters of urine from home to the lab, and for storing at home during the collection periods.
7. Labeling system of color-coded and numbered sample collection vials were devised, and vials were labeled.
8. Randomized ID numbering system was devised, so that at each visit, the patients received new ID numbers. This was done to increase blindedness of sample analysis by laboratory personnel.

Task 4. Subject Recruitment and Study, Months 5-10

a. Recruitment of healthy volunteers and selection of eligible subjects is estimated to take 2 months.

1. Recruitment into the study has been delayed for two reasons:
1) the fact that the work in the early phases was very labor-intensive and seasonally dependent; and
2) we wished to use information from another study to inform the dosing regiment of this study.
Originally, we had described a study in which there would be 6 clinic visits, but actually there would be 13 (including the crossover from seaweed/placebo to placebo/seaweed). The first year of this study would have been to look at the effects of escalating doses of seaweed on healthy women. In the other grant, funded by the Susan G. Komen Foundation, we planned to look at the effect of a single dose of seaweed on the same variables, and to monitor for any side effects. So, we decided to wait until the results of Komen study were known before starting this study of the effects of escalating doses. This would enable us to utilize the budget of this study most efficiently by tailoring the analyses to be only those that showed the most variation with seaweed.

2. Our initial recruiting strategies were cumbersome. In May, I was interviewed on a Worcester radio talk show and by a Boston-based TV news station. Because we didn't recruit enough subjects in May to begin the study, we used other strategies. We mailed a letter of invitation to every current and past breast cancer patient in the U Mass hospital system, made visits to breast cancer support groups, and recruited via word of mouth referrals from people who had been in previous studies. For healthy women, we used lists of women who had had negative mammograms, and mailed letters of invitation. On average, we recruited 1/100 letters sent out. Finally, after several months of effort, the local newspaper (Worcester Telegram and Gazette) to write a story about the difficulties of recruiting women for breast cancer prevention studies, and to use our study as an example. We easily got enough healthy volunteers, but finally had to accept that 16 women with early breast cancer was the most we could recruit. Since we wanted to start everyone together, and women lost interest after a few weeks or months or waiting to begin, we ran our

preliminary study with 36 women, (20 healthy women, and 16 women who had been treated for early breast cancer).

3. In our preliminary study (Komen funded), we wanted to start everyone at the same time point in order to ensure that the effect seasonal variations would be minimized. This created tremendous problems for potential volunteers. Most of the volunteers recruited in June were no longer interested/available in October. Based on our preliminary data, season was not an important variable. In this study, volunteers will begin our study within 2 weeks of initial contact.

b. Study will last 14 weeks for each of the 20 subjects.

1. There is no change of plan for this. The study will last 20 weeks for each subject. There will be two doses of seaweed, 3 g/d and 5 g/d, and the subjects will come in for clinic visits at baseline, at 3 weeks (after 3 weeks on 3 g/d), at 6 weeks (after 3 weeks of 5 g/d), at 7 weeks (after 5 g/d seaweed and 2 mg isoflavone/kg body weight), at 10 weeks (after 3 weeks washout period), at 13 weeks (after 3 weeks on 3 g/d) at 16 weeks (after 3 weeks of 5 g/d), at 17 weeks (after 1 week of 5 g/d and 2 mg isoflavone/kg body weight) and at 20 weeks (after 3 weeks washout period).
2. The number of subjects will depend on the cost of the analyses to be done. This will be based on which of the variables who significant variation as a result of seaweed supplementation in the pilot study.

Task 5. Data Analysis of Results from Healthy Volunteers, Months 11-12

a. Meetings with oncologists to present preliminary data.

1. Meetings will take place as soon as the data are available.

b. Final meeting with volunteers to explain study results and to answer any questions.

1. Meeting is scheduled for September, by which time all the analyses should be completed.

c. Annual report to USARMC

1. This is the annual report to USARMC.

Key Research Accomplishments

1. Expanded the scope of the research to include soy plus seaweed synergism.
2. Widened range of scientific questions to be asked from the data, and increased the number of collaborators.
3. Several additional analyses were added to the pilot study, in order to pinpoint areas where seaweed might be biologically active. These include: melatonin, arsenic, insulin-like growth factor 1 and insulin-like growth factor binding protein 3, a range of carotenoids including fucoxanthin, a carotenoid specific to brown seaweeds, estrogen metabolites, cancer cell inhibition studies, and neurotensin. Additional funding for these studies has been obtained from Our Danny Fund (a University of Massachusetts Cancer Center granting fund) and the LINK laboratory at the University of Massachusetts. Individual researchers have contributed their time and resources to pursue these interests, and we have been able to contribute some money by not having a secretary and by doing the laboratory processing ourselves. Results are pending.
4. Visited four seaweed harvesters to learn how seaweed is collected, and identified potential areas of contamination.
5. Assayed over 30 kinds of seaweed to find the lowest iodine-containing brown seaweed which would be safe for human consumption.
6. Identified and visited an encapsulator to make sure seaweed and placebo capsules would be of the highest quality.
7. Identified a recruitment strategy that was effective. Devised patient incentives that kept drop out (due to lack of interest) to 1 of the 36 volunteers.

Reportable Outcomes

None yet. The first analyses were available June 1, and analyses are not yet complete.

Conclusions

1. At this point, we know that taking 10 capsules of a brown seaweed, Alaria esculenta, did affect thyroid function and does not appear to affect melatonin function.
2. Preliminary work suggests that fucoxanthin, a carotenoid specific to brown seaweeds, appears to have been absorbed by the women in our study.
3. Specific results of most of the analyses are not yet available.

References

No published papers yet.

Appendices

None.

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