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Evaluation of Chemoprevention of Oral Cancer With *Spirulina fusiformis*

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Abstract

The blue-green microalgae Spirulina, *used in daily diets of natives in Africa and America, have been found to be a rich natural source of proteins, carotenoids, and other micronutrients. Experimental studies in animal models have demonstrated an inhibitory effect of* Spirulina *algae on oral carcinogenesis. Studies among preschool children in India have demonstrated* Spirulina fusiformis *(SF) to be an effective source of dietary vitamin A. We evaluated the chemopreventive activity of SF (1 g/day for 12 mos) in reversing oral leukoplakia in pan tobacco chewers in Kerala, India. Complete regression of lesions was observed in 20 of 44 (45%) evaluable subjects supplemented with SF, as opposed to 3 of 43 (7%) in the placebo arm ($p < 0.0001$). When stratified by type of leukoplakia, the response was more pronounced in homogeneous lesions: complete regression was seen in 16 of 28 (57%) subjects with homogeneous leukoplakia, 2 of 8 with erythroplakia, 2 of 4 with verrucous leukoplakia, and 0 of 4 with ulcerated and nodular lesions. Within one year of discontinuing supplements, 9 of 20 (45%) complete responders with SF developed recurrent lesions. Supplementation with SF did not result in increased serum concentration of retinol or β-carotene, nor was it associated with toxicity. This is the first human study evaluating the chemopreventive potential of SF. More studies in different settings and different populations are needed for further evaluation.*

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Introduction

Oral leukoplakia is a well-established precancerous lesion. The nonhomogeneous lesions (erythroplakia, nodular/ulcerated leukoplakia, verrucous leukoplakia) are at particularly high risk for oral cancer (1). Spontaneous regression is associated with lesions demonstrating hyperkeratosis (2); these are mostly homogeneous leukoplakias. Spontaneous regression is uncommon among nonhomogeneous lesions. Several studies of chemoprevention of oral cancer have used oral leukoplakia as an end point (3–7). There is considerable interest in the chemoprevention of oral cancer on three accounts: 1) it has potential as a complementary

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strategy in the prevention of oral cancer, 2) subjects at high risk for oral cancer could be identified, and 3) retinoids and carotenoids have been shown to reverse oral precancerous lesions, even in the presence of continuing carcinogenic exposure.

The rationale behind chemoprevention comes from the finding from epidemiologic studies that a diet rich in vegetables and fruits protects against several epithelial cancers and from the evidence from experimental animal studies demonstrating the protective effect of suspected micronutrients (8–10). Perhaps several substances in these food items, the actions of which could be as complex as the carcinogenic process, are responsible for the protective effect, rather than a single or a few micronutrients. Evidence from observational epidemiologic studies indicates that consumption of certain food items is associated with reduced risk of cancer. Food items like the blue-green algae *Spirulina fusiformis* (SF), which are rich in several micronutrients, have been studied in animal models for their ability to suppress carcinogenesis (11–13). This prompted us to evaluate the potential of these blue-green algae in the chemoprevention of oral cancer. To our knowledge, this is the first human study involving SF in chemoprevention.

Materials and Methods

Sixty subjects with oral leukoplakias were recruited from the fishing villages of Trivandrum, Kerala, India. They were given oral SF (lyophilized powder, 1 g/day for 1 yr). The supplies of a wholesome unaltered sterile original virgin form of SF were provided by New Ambadi Estates (Madras, India). Fifty-five subjects were randomized to the placebo arm as part of another study to evaluate the chemopreventive potential of β-carotene and vitamin A. The placebo capsules were provided by Hoffmann-LaRoche (vehicle used in the preparation of β-carotene beadlets). These subjects were used as the control group for comparison in this study as well. All subjects were ethnically and socioeconomically comparable Latin Catholic Christians from the fishing villages around Trivandrum.

Forty-four subjects in the intervention arm and 43 in the placebo group continued in the study for one year, and further discussion relates to these compliant subjects only. The characteristics of compliant subjects are shown in Table 1. They regularly reported for review examination and for refill of capsules. Subjects were given supplies lasting for one month, and refills were provided after compliance with the regimen was ensured. More than 95% of the complaint subjects did not have leftover supplies at the time of refill.

Details such as habits and number, type, and dimension of lesions were recorded at baseline and at review. Biopsies from the lesions and blood samples were collected at baseline and at completion of supplementation at 12 months. Histological examination of the biopsy samples was performed by an oral pathologist to rule out malignancy and to establish the histological nature of lesions; the baseline histological characteristics are shown in Table 1. Eight of 43 (18.6%) subjects in the control group had mild dysplasia and 2 of 43 (4.7%) had moderate dysplasia. Eight of 44 (18%) subjects in the intervention group had mild dysplasia. Serum hemoglobin, proteins, retinol, α-tocopherol, χ-tocopherol, β-carotene, α-carotene, lutein/zeaxanthine, cryptoxanthine, and lycopene were measured at baseline and at completion of supplementation. The subjects were advised to give up their tobacco and alcohol habits at recruitment and at reviews.

The subjects were visited once every two months by a dentist and a physician, who were blinded to treatment groups. Using a questionnaire, they assessed the response of the lesions and inquired about the habits and side effects suffered by the subjects. Response was determined by the number and dimensions of the lesions at baseline and at the end of supplementation at 12 months. A complete response (CR) was defined as the total disappearance of the lesion(s), as assessed by visual inspection. A partial response was defined as ≥50% decrease in the size of single lesions or in the sum of dimensions of multiple lesions. Stable and

Table 1. Subject Characteristics		
Characteristic	Placebo ($n = 43$)	*Spirulina Fusiformis* ($n = 44$)
Males:females	28:15	38:6
Mean age, yrs	48.7	45.6
Chewers[a]	42 (21.8)	42 (24.6)
Smokers[a]	13 (6.0)	15 (6.7)
Drinkers[a]	21 (10.1)	31 (17.8)
Homogeneous leukoplakia	27	28
Nonhomogeneous leukoplakia	16	16
Baseline hemoglobin, g/100 ml	13.7	12.3
Mean albumin, g/100 ml	3.7	3.7
Mean globulin, g/100 ml	2.9	3.0
Pathological characteristics,[b] %		
Thickening of epithelium	38	42
Keratinization	80	93
Basilar hypertrophy	47	61
Loss of polarity	38	54
Anisocytosis	34	43

a: Values in parentheses represent mean duration of habits in yrs.
b: Proportion of subjects with moderate-to-severe criteria.

progressive lesions were scored as "no response." Lesions suspicious for malignancy were biopsied during reviews.

The differences in response between the placebo and intervention arms were tested by χ^2 test. The differences in the serum levels of micronutrients at baseline and completion of supplementation were tested by Wilcoxon rank sum test. All tests were two-tailed, and $p < 0.05$ conferred statistical significance.

Results

The CR rates of homogeneous lesions were 11% and 57% with placebo and SF, respectively ($p < 0.001$); 0% and 25% were the corresponding rates for nonhomogeneous lesions ($p = 0.051$; Table 2). None of the nodular and ulcerated leukoplakias ($n = 4$) responded to therapy with SF; two of eight subjects with erythroplakia and two of four with verrucous leukoplakia had CR of the lesions with SF.

The CR rates were 46% (17 of 37) for lesions ≤2 cm in maximum dimension with SF; CR was 43% (3 of 7) for lesions >2 cm in diameter. Two of the eight subjects (25%) with dysplasia had CR after supplementation with SF.

Two of 43 subjects in the placebo arm had malignant transformation compared with 1 of 44 in the study group during supplementation. Five and seven subjects complained of frequent headache and muscular pain after the SF intake. No such complaints were observed in the

Table 2. Response of Lesions After 12 Months of Supplementation[a]				
	Placebo		*Spirulina* algae	
Response	Homogeneous ($n = 27$)	Nonhomogeneous ($n = 16$)	Homogeneous ($n = 28$)	Nonhomogeneous ($n = 16$)
Complete	3 (12%)	0	16 (57%)	4 (25%)
Partial	0	0	4 (14%)	1 (6%)
Nil	23 (88%)	14 (83%)	8 (29%)	10 (63%)
Malignant transformation	0	2 (17%)	0 (0%)	1 (6%)

a: Responses were tested for significant difference in outcome after 12 mos of supplementation: homogeneous lesions: $p < 0.0001$; nonhomogeneous lesions: $p = 0.051$.

Table 3. Mean Serum Levels of Micronutrients at Baseline and at Completion of Supplementation[a]

Factor	Placebo		*Spirulina* algae	
	Baseline	End Point	Baseline	End Point
Retinol, μg/dl	83.5	65.5	65.1	65.6
β-Carotene, μg/ml	0.120	0.098	0.098	0.096
α-Carotene, μg/ml	0.025	0.017	0.026	0.017
Lutein/zeaxanthine, μg/ml	0.23	0.17*	0.22	0.16*
Cryptoxanthine, μg/ml	0.18	0.12†	0.21	0.16
Lycopene, μg/ml	0.015	0.013	0.0	0.0
α-Tocopherol, μg/dl	759.8	680.2	722.2	700.0
χ-Tocopherol, μg/dl	0.0	32.2†	0.0	0.0

a: Statistical significance is as follows: *, $p < 0.05$; †, $p < 0.01$.

placebo group. Within one year after stopping supplementation, 9 of 20 (45%) subjects with CR in the SF arm reported with recurrence of lesions. Among the nonresponders, one exhibited malignant transformation during the second year. Two more subjects in the placebo arm revealed malignant transformation during the second year. Thus, at two years follow-up, malignant transformations were observed in 4 of 43 (10%) subjects in the placebo group and 2 of 42 (5%) subjects in the study group.

The mean plasma retinol levels were 83.4 and 65.1 ng/dl in the placebo and SF arms, respectively, at baseline; the corresponding levels after 12 months of supplementation were 65.1 and 65.6 μg/dl (Table 3). The mean β-carotene values at entry were 0.12 and 0.098 μg/ml in the placebo arm and β-carotene arm, respectively; the respective values at one year were 0.098 and 0.096 μg/ml. The baseline mean α-tocopherol levels were 759.8 and 722.2 μg/dl in the placebo arm and SF arm, respectively; the corresponding values at the end of one year were 680.2 and 700.3 μg/dl, respectively.

Discussion

Spirulina algae have a higher content of β-carotene and other carotenoids than any other plant source. *Spirulina* algae are found in highly alkaline lakes of Africa, Mexico, and Asia, where the natives have been including this item in their daily diets for centuries (14). It has been demonstrated to be a safe food item and could be cultivated throughout the year; in fact, commercially viable processes for outdoor cultivation of *Spirulina* have been developed (15).

The micronutrient and chemical composition of the SF provided to us for supplementation was as follows: 4 mg/g total carotenes, 2 mg/g natural β-carotene, 0.06 mg/g vitamin B complex, 1 μg/g cyanacobalamin, 1 mg/g iron, 8.5 mg/g phosphorus, 0.35 mg/g zinc, and 13.5 mg/g potassium (15). The chemical composition was 9.0% water, 55.0% proteins, 0.9% crude fiber, and 9.0% ash. The conditions of storage of SF have been reported to affect their micronutrient content. Carotene losses are significantly lower when SF is stored in cold room conditions than at room temperature.

The epidemiologic evidence of the protective effect of diets rich in vegetables and fruits and the evidence from experimental animal studies and small human intervention studies indicating the inhibitory effect of micronutrients, such as carotenoids, retinoids, and vitamin E, on carcinogenesis indicate that SF might be a potential chemopreventive food item. Realization of the preventive potential of micronutrients should preferably be achieved by modulation of diet rather than in the form of pharmacological supplements. The purpose of this study was to explore whether SF has any demonstrable clinical activity in reversing oral precancerous lesions.

SF has been demonstrated to be an effective dietary source of vitamin A. An investigation

in India among preschool children with vitamin A deficiency demonstrated that the bioavailability of carotenes from SF is comparable to that from other sources such as carrots and green leafy vegetables, thereby suggesting the potential use of SF as a dietary source of vitamin A (16). The absorption of total carotenes and β-carotene from a single bolus dose of SF containing 1,200 μg of β-carotene after a seven-day carotene-free diet was examined in three- to five-year-old preschool children. The percent absorption of total carotenes varied from 55.7 to 88.9 of the total carotene ingested from SF; the percent absorption of β-carotene ranged from 63.5 to 86.8 of the ingested amount of 1,200 μg from 2 g of SF.

The effect of daily supplementation of vitamin A (300 μg/day for 1 mo) in 25 preschool children or SF (2 g/day for 1 mo) in 32 children on the serum concentration of retinol was studied in South India (16). The serum retinol level significantly improved from the baseline level of 21.4 ± 6.23 to 30.3 ± 6.88 μg/dl in children supplemented with SF; the corresponding measurements were 22.9 ± 6.71 and 34.8 ± 7.66 μg/dl with vitamin A. The increase was greater in children with baseline retinol levels <20 μg/dl: from the baseline mean level of 14.8 ± 3.47 to 29.3 ± 6.89 μg/dl. On withdrawal of the supplements, serum retinol levels returned to their presupplementation levels within one to three months. It was concluded that SF could be used as a dietary source of vitamin A.

We did not observe an increase in the mean level of plasma retinol one year after supplementation with SF (Table 3). Our subjects had normal plasma concentration of vitamin A at baseline and did not suffer from vitamin A deficiency. Nor did we observe an increase in the serum concentration of β-carotene after supplementation. This is partly attributable to the small contribution of β-carotene from SF (<2 mg/day). Although the dose of β-carotene is very much lower than the dose employed in other studies (15–90 mg/day), SF provided other natural constituents, including carotenoids, vitamin E, other micronutrients, and proteins in their natural state without any extraction procedure. The unchanged serum end point values of vitamin A, carotenoids, and α-tocopherol indicate a possible synergistic effect between these various ingredients of the algae extract. The algae extract has been shown to be more effective than single nutrients, like β-carotene or canthaxanthine, in inducing regression of 7,12-dimethylbenz[*a*]anthracene-induced hamster buccal pouch cancers (11). Significant synergistic chemoprevention of oral cancers by a mixture of antioxidants like β-carotene and α-tocopherol has been demonstrated in animal models (12). In this regard, the contribution of other plant pigments belonging to the family of phycocyans and phycoerythrins should be taken into consideration when one evaluates the total cancer chemopreventive potential of natural products.

A long-term program on dietary supplementation of SF to 5,000 preschool children through the integrated child development scheme was initiated in Pudukottai District, Tamil Nadu State, South India, in 1991, and the initial results reveal general improvement in health (15). Many health effects of SF were recently reviewed in an Ecology, Taxonomy, Technology and Applications (ETTA) symposium on *Spirulina* organized by the Departments of Science and Technology and Biotechnology of the Government of India (15). The effects claimed include antioxidant activity, because of the high content of superoxide dimutase and β-carotene, and improvements in iron content and hypocholesterolemic and hypoglycemic effect. The human studies describing the above effects did not show toxicity with supplementation.

Spirulina extracts have been shown to inhibit buccal cancers in animal studies (11–13). An extract of *Spirulina* and *Dunaliella* algae was shown to prevent tumor development in the hamster buccal pouch (11). The same extract resulted in regression of 7,12-dimethylbenz[*a*]anthracene-induced hamster buccal squamous cell carcinomas (12).

The major outcome from this trial is that a clinical response with SF in reversing oral leukoplakias has been demonstrated, and no serious toxicities were observed during or after supplementation. The response was more pronounced with small and homogeneous lesions. Limited response was observed for erythroplakia and verrucous leukoplakia, but the sample

size was small. More human trials with hard end points in different settings and different populations are required to further establish the effectiveness of *Spirulina* algae before any conclusions can be made. Such studies in oral cancer should involve high-risk nonhomogeneous leukoplakias or the dysplastic lesions. If the chemopreventive potential could be established by such studies, *Spirulina* algae could be easily and acceptably incorporated into daily dietary practices.

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