β-Carotene Supplementation Results in an Increased Serum and Colonic Mucosal Concentration of β-Carotene and a Decrease in α-Tocopherol Concentration in Patients with Colonic Neoplasia[1]

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Abstract

The aim of this study was to evaluate the colonic mucosal β-carotene (BC) concentration following supplementation with BC and to determine if an increase in BC concentration influences vitamin E (α-tocopherol) status. The concentration of BC and α-tocopherol was assessed in serum and colonic tissue obtained from subjects with a history of colonic polyps or resected cancer (Dukes A, B_1, or B_2). Serum and mucosal biopsy samples were obtained prior to and following 3 months daily p.o. supplementation with 30 mg of BC or placebo. The concentration of BC was significantly increased in serum and colonic mucosa from both polyp and cancer subjects following supplementation as compared to presupplementation values and values from subjects receiving a placebo. The concentration of α-tocopherol in serum from cancer subjects was significantly decreased in samples obtained at the end of 3 months of BC supplementation as compared to placebo-matched controls. In BC-supplemented polyp subjects the tissue concentration of α-tocopherol was also significantly decreased relative to presupplementation values. The results indicate that BC supplementation does result in a significant accumulation of BC in the colonic mucosa but that the α-tocopherol concentration in both serum and colonic tissue may be compromised by an increased intake of BC. The mechanism for the decrease in α-tocopherol in conjunction with the increase in BC will require further study in order to develop strategies which will prevent vitamin E deficiency in BC-supplemented individuals.

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Introduction

Results from experimental and epidemiological studies suggest that BC[3] protects against the development of various human cancers (1). For example, a number of studies have concluded that decreased consumption of BC and low serum BC concentration is correlated with an increase risk of lung cancer (2, 3). Data establishing an association between relatively decreased serum BC concentration or BC intake and a reduction in the risk of cervical (4) and stomach cancer (5) as well as oral leukoplakia (6) have also been reported. In addition, BC has also been shown in *in vitro* studies to protect against carcinogen-induced transformation of mouse fibroblasts (7) and to protect against chemical carcinogens in experimental animals (8). Collectively these data have provided the basis for a more extended analysis of the effect of BC in protecting against the initiation and progression of various human cancers in controlled clinical trials (9). In a recent report containing data from an intervention trial in China, BC in combination with α-tocopherol and selenium was reported to reduce cancer mortality (10).

The primary effect of BC in preventing carcinogenesis is thought to be due to its antioxidant activity. However, other mechanisms, including an inhibition of cellular proliferation and enhancement of various immune functions, have also been attributed to BC or products of BC metabolism (11). The protective effect of BC may prove to involve a combination of modulatory events which together retard the initiation and growth of cancer cells.

The use of BC as a chemopreventive agent is considered advantageous relative to other carotenoids as well as retinoids by virtue of its low cost and availability in a variety of dietary products. In addition, the antioxidant effect of BC is unique in that it is particularly effective at low oxygen pressures (12). Although BC has not previously been shown to have detrimental effects, a recent study conducted using Finnish men with a chronic, heavy addiction to cigarette smoking concluded that a slightly increased incidence of lung cancer occurred in those receiving BC supplementation relative to those receiving a placebo (13). Interestingly, despite the provitamin A activity of BC, vitamin A toxicity does not result from consumption of BC. However, BC may have an antagonistic effect on the concentration of other antioxidants, specifically α-tocopherol. In a recent study, Xu *et al.* (14) have reported that BC decreases the serum and skin levels of α-tocopherol in humans. Thus, the concentration of α-tocopherol, which is effective as an antioxidant

[3] The abbreviations used are: BC, β-carotene; HPLC, high performance liquid chromatography.

Table 1 Demographic characteristics of study participants[a]

Group	Male			Female			Total		
	No.	Age (yr)	Range	No.	Age (yr)	Range	No.	Age (yr)	Range
Polyp									
Total	19	59.2 ± 6.8	46–69	15	57.0 ± 12.0	40–80	34	58.2 ± 9.5	40–80
Placebo	10	59.2 ± 4.6	50–66	8	57.6 ± 12.2	41–78	18	58.5 ± 8.9	41–78
BC	9	59.2 ± 8.5	46–69	7	56.3 ± 11.7	40–80	16	57.9 ±10.2	40–80
Cancer									
Total	19	68.5 ± 8.7	45–83	5	69.2 ± 6.9	60–78	24	68.9 ± 8.4[a]	45–83
Placebo	9	64.8 ± 8.9	45–77	3	71.3 ± 8.0	60–78	12	66.4 ± 9.1	45–78
BC	10	71.8 ± 7.0	55–83	2	66.0 ± 2.0	64–68	12	70.8 ± 6.8	55–83

[a] The age of subjects in the cancer group is significantly greater than the age of subjects in the polyp groups ($P < 0.01$).

at relatively higher oxygen pressures (12), may be compromised by increasing the BC concentration.

In keeping with our interest in colorectal cancer which represents the second most common cancer in the United States with over 160,000 new cases reported each year, the present study was designed to measure the concentration of BC in colonic mucosal cells as well as serum obtained from individuals receiving BC supplementation. The hypothesis for this study is that p.o. supplementation in moderate doses will significantly increase the serum and colonic mucosal BC concentration and will not significantly compromise the level of a second important antioxidant vitamin, α-tocopherol, in colonic mucosa. The data reported here are the initial results from a chemoprevention trial to determine the effects of BC on intermediate biochemical endpoints.

Materials and Methods

Subjects and Study Design. Subjects (age ≥40 years) responding to advertisements were recruited for the study following an initial standard history and physical examination. All subjects had experienced a colonic polyp or cancer which had been diagnosed within the past 5 years. Criteria for inclusion into the study included an analysis of the results from a colonoscopy performed within 2 years prior to recruitment which confirmed no recurrence of colonic polyps or tumors. Subjects with diabetes and those administered antibiotics, vitamin supplements, or nonsteroidal antiinflammatory drugs at the time of admission to the study were excluded. Subjects with a previous history of colonic cancer (Dukes A, B_1, or B_2) were included if no chemotherapy or radiation therapy was performed as part of their treatment. All subjects were asked to sign a consent form approved by the Institutional Review Board for use of Human Subjects at Loyola University Medical Center.

Tissue and blood samples were obtained on day 0, at which time subjects were provided with 90 capsules each containing either a placebo or 30 mg BC. BC was kindly provided by Hoffmann LaRoche Inc. (Nutley, NJ) through the office of Ogden Bioservices Corporation. Subjects were instructed to take one capsule each day and were contacted on a periodic basis to enhance compliance. After 3 months subjects returned and additional rectal mucosal tissue and blood samples were obtained. Colonic mucosal tissue was obtained by unprepped sigmoidoscopy in the endoscopy facility at Loyola University. Tissue samples were placed in normal saline and stored at −70°C. Blood samples were collected in vacutainer tubes which were centrifuged and the serum was removed for storage at −70°C. Subjects were selected to receive placebo or BC on a random basis and with the exception of the individual allocating capsules, all individuals participating in the study were blind as to which subject received BC. Analysis of tissue and serum samples was performed on material from 16 polyp subjects receiving BC, 18 polyp subjects receiving placebo, 12 cancer subjects receiving BC, and 12 cancer subjects receiving placebo. Nine subjects did not complete the study and data from these subjects is not included in the analysis. The demographic characteristics of subjects who completed the study are provided in Table 1.

Analysis of BC and α-Tocopherol. Quantitation of BC and α-tocopherol concentration in tissue and serum samples as well as retinol concentration in serum was performed by HPLC as described previously (15). Briefly, serum samples were deproteinized with ethanol and extracted twice with hexane containing butylated hydroxytoluene (0.01% w/v). Samples were then evaporated to dryness and the residue was reconstituted in diethyl ether and a quantity of mobile phase to bring the sample to its original volume. Tissue samples were homogenized and saponified by the addition of 10 N KOH and pyrogallol:methanol (1.0 g/50 ml), followed by heating at 65°C for 60 min. After repeated extraction with hexane, the sample was dried and then reconstituted as described. Samples were analyzed by HPLC using a 490 programmable multiwavelength detector after resolution of peaks on a Nova-pak C_{18} column. The saponification necessary for extraction of α-tocopherol and BC precluded extraction and quantitation of retinol in tissue samples. However, serum retinol was determined for each subject. The coefficients of variations for measurement of serum BC and serum α-tocopherol were 6.4 and 3.2%, respectively. The individual performing the HPLC analysis of tissue and serum samples was blind as to the group and supplementation provided to each subject.

Statistical Analysis. The Student's *t* test and the χ^2 test were used to compare mean age and gender composition, respectively, between the two groups. Differences between values for baseline and 3 months within the same group were examined by paired *t* test. Analysis of covariance was used to compare mean change from baseline to 3 months in subjects receiving BC to those receiving placebo with adjustment for presupplementation values. The results were similar when nonparametric procedures (Wilcoxon signed ranks test and Mann-Whitney *U* test) as well as log transformation of data were performed.

Table 2 Serum β-carotene and α-tocopherol concentrations for polyp and cancer subjects receiving placebo or BC. Analysis of serum samples collected at 0 and 3 months

Group	No.	Month	BC or Placebo	α-Tocopherol (μg/dl)	BC (μg/dl)
Polyp	18	0	Placebo	1274.9 (± 462.7)	18.7 (± 16.0)
		3		1168.1 (± 350.7)	19.0 (± 13.8)
Polyp	16	0	BC	1282.7 (± 434.5)	20.4 (± 12.8)
		3		1267.3 (± 276.8)	238.0[a, b] (± 153.7)
Cancer	12	0	Placebo	1168.7 (± 290.4)	15.8 (± 13.8)
		3		1341.6[b] (± 289.8)	16.9 (± 15.2)
Cancer	12	0	BC	1058.7 (± 265.6)	14.4 (± 9.6)
		3		993.1[a] (± 192.0)	178.1[a, b] (± 81.1)

[a] Significant difference in change from 0 to 3 months compared to values obtained for individuals receiving placebo with adjustment for 0 month values for BC ($P < 0.001$) and α-tocopherol ($P < 0.01$).
[b] Values are significantly different from 0 to 3 months for subjects within groups ($P < 0.01$).

Results

Subjects with a previous colonic tumor that were recruited for the study were significantly older as a group than polyp subjects (Table 1). In general, more men responded to advertisements than women with the greatest sex-based discrepancy in respondents occurring in the cancer group. There was, however, no difference between the groups in regard to gender composition. There were no reported cases of toxicity in subjects receiving BC with the exception of one individual who was dropped from the study after complaining of mild stomach discomfort. Eight other subjects did not complete the study. Compliance in regard to capsule intake was excellent (range, 90–100% of pills used by all study subjects).

Baseline serum BC concentrations for the cancer and polyp groups were not significantly different (19.5 ± 14.4 (SD), $n = 34$ and 15.1 ± 11.7 μg/dl, $n = 24$, respectively). There was, however, a significant difference in the serum α-tocopherol concentration at baseline between the polyp and cancer groups (1278.6 ± 442.9 μg/dl and 1116.1 ± 278.2 μg/dl for polyp and cancer groups, respectively; $P = 0.02$). The comparison for cancer subjects receiving BC to cancer subjects receiving placebo indicates a significant difference in the change from 0 to 3 months for serum α-tocopherol concentration ($P < 0.01$) in addition to the significant increase in BC concentration ($P < 0.001$) (Table 2). The difference in the change in serum α-tocopherol concentration between cancer subjects receiving placebo and BC was primarily due to a significant increase (+173 μg/dl) in the placebo group in comparison to the relatively minor decrease (−65 μg/dl) in the BC-supplemented group. A significant difference in the change in serum BC concentration between subjects receiving placebo and BC was also observed in the polyp group ($P < 0.001$). In all subjects receiving BC, the serum BC concentration was elevated at 3 months when compared to values from 0 months, further indicating excellent patient compliance. The serum BC concentration increased 12.5- and 10.5-fold in samples from polyp and cancer subjects, respectively, compared to subjects receiving placebo. The serum retinol concentration was not significantly different for the two groups at baseline (60.8 ± 18.7 and 63.4 ± 15.0 μg/dl for polyp and cancer subjects, respectively) and was not altered by supplementation with BC (65.6 ± 15.6 and 62.8 ± 15.5 μg/dl, respectively). However, there was a significant increase in the serum retinyl ester concentration in polyp subjects receiving BC (21.7 ± 12.9 post-BC *versus* 7.2 ± 5.5 μg/dl pretreatment; $P < 0.05$). There was also an increase in retinyl ester concentration in serum of cancer subjects following BC supplementation but the increase was not significant (16.0 ± 13.7 post-BC *versus* 6.5 ± 4.8 μg/dl).

The tissue concentration of BC and α-tocopherol in samples from polyp and cancer groups was not significantly different at 0 months (91.3 ± 74.8 and 91.3 ± 110.5 ng/g of tissue for BC and 4.7 ± 3.0 and 3.3 ± 1.6 μg/g of tissue for α-tocopherol for polyp and cancer groups, respectively). A significant increase in the BC concentration from baseline to 3 months was determined for samples from both polyp and cancer subjects supplemented with BC relative to those receiving placebo ($P < 0.001$) (Table 3). Tissue α-tocopherol concentration was also significantly different in BC-supplemented polyp subjects from 0 to 3 months relative to the change in those receiving placebo ($P < 0.05$). In cancer subjects there was a greater decrease in tissue α-tocopherol concentration from 0 to 3 months in those receiving BC relative to those receiving placebo but the difference did not reach statistical significance. The increase in tissue BC concentration from 0 to 3 months was 13- and 7.5-fold for polyp and cancer subjects receiving BC, respectively.

Discussion

The data from a number of epidemiological studies suggest that BC protects against the initiation of events which lead to cancer in humans (1). As a result of these studies, the efficacy of BC in the prevention of human cancer is currently being assessed in a number of clinical trials. The antioxidant activity of BC is hypothesized to be the mechanism by which BC protects against the development of cancer (11). However, the possibility exists that BC is metabolized to retinoids and it is these metabolites which ultimately function to prohibit the harmful effects which can occur during oxidative stress. Recently it has been shown *in vitro* that treatment with BC as well as other carotenoids increases the mRNA and protein expression of the gap junctional protein connexin43 (16). Thus, a change in cellular communication resulting from BC treatment represents a third possible mechanism by which BC protects against the onset of cancer.

Although BC is thought to be a cancer chemopreventive agent, there have been few reports from human trials in

Table 3 Tissue concentrations of β-carotene and α-tocopherol for polyp and cancer groups receiving placebo or BC Analysis of tissue collected at 0 and 3 months

Group	Month	BC or Placebo	α-Tocopherol (μg/g)	BC (ng/g)
Polyp	0	Placebo	4.0 (± 2.4)	104.7 (± 92.1)
	3		4.1 (± 3.1)	89.5 (± 51.0)
Polyp	0	BC	5.7 (± 3.5)	77.9 (± 52.0)
	3		2.7 (± 1.2)[a]	1025.6 (± 736.1)[a, b]
Cancer	0	Placebo	3.9 (± 1.3)	89.7 (± 64.2)
	3		3.8 (± 1.9)	75.4 (± 37.4)
Cancer	0	BC	2.6 (± 1.7)	93.1 (± 148.8)
	3		2.1 (± 2.0)	699.4 (± 373.5)[a, b]

[a] Values are significantly different from 0 to 3 months for subjects within groups for BC ($P < 0.001$) and α-tocopherol ($P < 0.05$).
[b] Significant difference in change from 0 to 3 months as compared to values obtained for individuals receiving placebo with adjustment for 0 month values ($P < 0.001$).

which the concentration of BC has been demonstrably increased in a specifically targeted tissue. In a recent study, BC concentration was quantitated in the rectal mucosa from 20 cancer subjects receiving BC supplementation (17). Although this study was not placebo controlled and included only individuals with a prior history of colonic cancer, the results did indicate that BC taken p.o. does accumulate in the rectal mucosa. In view of the fact that colon cancer represents the second most common cancer in the United States, in this study we elected to determine the concentration of BC in colonic tissue as well as serum of individuals with a prior history of colonic polyps or cancer in a placebo-controlled study. Our analysis of serum and tissue included quantitation of α-tocopherol in order to determine the effect of BC supplementation on the concentration of this important antioxidant vitamin.

The results of this study indicate a highly significant increase in both serum and colonic mucosal BC concentration in individuals supplemented daily with 30 mg BC. The observed increase in serum BC concentration is consistent with that reported by others for individuals supplemented with BC (18, 19) and the serum and tissue BC concentration for cancer subjects is similar to that reported recently for cancer subjects receiving BC supplementation (17). The average BC concentration in serum of polyp and cancer subjects was 22 and 63% lower, respectively, than values reported for individuals supplemented for 18 months (20). The BC serum concentration in polyp subjects following supplementation was slightly lower (13%) than previously reported for healthy individuals supplemented for 3 months with 20 mg BC (20). However, in cancer subjects the quantity of BC present in serum was 50% less than that measured for individuals on the 3-month/20-mg dose regimen. Together these results suggest that a 3-month period at a 30-mg/day dosage is sufficient to reach very high and possibly near maximal serum BC levels in polyp subjects. The situation with cancer subjects appears to be more complex. A lower serum and tissue BC concentration was evident for cancer subjects relative to polyp subjects, although as previously stated the serum and tissue BC concentrations for cancer subjects were very similar to those reported earlier (17). The lower serum BC concentration in supplemented cancer subjects may in part reflect the difference observed in samples collected prior to supplementation and may possibly indicate some minor difference in diet between the two groups. Other factors, however, including the greater age of cancer subjects and the possible alteration in BC absorption resulting from colonic resection in cancer subjects, may account for the relative decrease in tissue BC concentration. Regardless of the mechanism, the data clearly indicate that the serum and tissue BC concentration attained in polyp subjects by supplementation is not achieved in individuals with a prior resected colonic tumor. At present, tissue and serum samples are being collected in order to determine the time interval following cessation of BC supplementation before values return to presupplementation levels. This evaluation, particularly in regard to tissue BC concentration, will serve in the development of treatment strategies.

The lower baseline serum α-tocopherol concentration in cancer subjects as compared to polyp subjects may indicate either lower intake or more rapid metabolism by cancer subjects. A number of human studies have concluded that serum α-tocopherol concentration increases with age (21, 22) and is not decreased in response to BC supplementation (21, 23, 24). The present results, although not conclusive in regard to the effect of BC on serum α-tocopherol concentration, suggest that BC supplementation may negatively affect the concentration of α-tocopherol in serum. The extent to which age affects the α-tocopherol concentration in response to BC supplementation has not been determined. It is, however, of interest to note that in those studies in which no change in α-tocopherol was determined, the average age of subjects was <40 (21, 23, 24), whereas in the study by Xu *et al.* (14) and the current study, the average age was >55. The current debate regarding the effect of BC supplementation on the concentration of other potential chemopreventative vitamins may be somewhat resolved by a more detailed analysis of the influence of age.

Although studies have not been performed on a sufficient number of human tissues, there may be tissue specificity in regard to a coincident decrease in α-tocopherol with an increase in BC concentration. α-Tocopherol is a significant antioxidant that is obtained from dietary sources and is also under study as a potential cancer chemopreventive agent (9). If an elevation in BC concentration in some as yet undescribed manner compromises the concentration of α-tocopherol, the overall effect would be to diminish the expected increase in antioxidant protection that would be

afforded by an increase in BC concentration alone. We found no change in serum retinol concentration in samples from subjects supplemented with BC. Thus, the apparent antagonism between BC and α-tocopherol does not appear to compromise the metabolism of retinol. Further study will determine if it is most advantageous to cosupplement individuals with BC and α-tocopherol in order to attain maximal antioxidant defenses. In a recent study, Blot *et al.* (10) reported that BC in combination with α-tocopherol and selenium reduced the incidence of mortality in the Linxian region of China, which has a very high rate of esophageal/gastric cancer. Although the antioxidant activity of BC could theoretically compensate for a decrease in α-tocopherol concentration, this compensation may not be sufficient owing to the relative incapacity of BC to function as an antioxidant at higher oxygen pressures (12).

In summary, the present data indicate that BC supplementation significantly increases the BC concentration in both serum and colonic mucosa of individuals having a previous colonic polyp or cancer. Serum α-tocopherol concentrations are significantly lower in cancer subjects relative to polyp subjects and the concentration of α-tocopherol is significantly decreased in the tissue of polyp subjects in response to the elevation in BC concentration. The extent to which BC prevents the onset of cancer in the colon remains to be demonstrated. However, it is clear that p.o. supplementation with BC does result in an accumulation of BC in the colonic mucosa.

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