Neoplastic and Antineoplastic Effects of β-Carotene on Colorectal Adenoma Recurrence: Results of a Randomized Trial

John A. Baron, Bernard F. Cole, Leila Mott, Robert Haile, Maria Grau, Timothy R. Church, Gerald J. Beck, E. Robert Greenberg

Background: In two large, randomized prevention trials, supplementation with β-carotene increased the risk of lung cancer. Subjects in these studies were predominantly cigarette smokers, and the adverse effects were concentrated among those who also drank alcohol. Although β-carotene supplementation appeared not to increase the risk of cancer generally, it is not clear if smoking and/or alcohol use alters the effect of β-carotene on carcinogenesis at sites outside the lung.

Methods: We studied the effect of β-carotene supplementation on colorectal adenoma recurrence among subjects in a multicenter double-blind, placebo-controlled clinical trial of antioxidants for the prevention of colorectal adenomas. A total of 864 subjects who had had an adenoma removed and were polyph-free were randomly assigned (in a factorial design) to receive β-carotene (25 mg or placebo) and/or vitamins C and E in combination (1000 mg and 400 mg, respectively, or placebo), and were followed with colonoscopy for adenoma recurrence 1 year and 4 years after the qualifying endoscopy. A total of 707 subjects had two follow-up examinations and provided smoking and alcohol use data. Adjusted multivariate risk ratios (RRs) and 95% confidence intervals (CIs) were used to assess the effects of β-carotene on adenoma recurrence.

Results: Among subjects who neither smoked cigarettes nor drank alcohol, β-carotene was associated with a marked decrease in the risk of one or more recurrent adenomas (RR = 0.56, 95% CI = 0.35 to 0.89), but β-carotene supplementation conferred a modest increase in the risk of recurrence among those who smoked (RR = 1.36, 95% CI = 0.70 to 2.62) or drank (RR = 1.13, 95% CI = 0.89 to 1.43). For participants who smoked cigarettes and also drank more than one alcoholic drink per day, β-carotene doubled the risk of adenoma recurrence (RR = 2.07, 95% CI = 1.39 to 3.08; \( P \) for difference from nonsmoker/non-drinker RR < .001). Conclusion: Alcohol intake and cigarette smoking appear to modify the effect of β-carotene supplementation on the risk of colorectal adenoma recurrence. [J Natl Cancer Inst 2003;95:717–22]

During the early 1980s, three large lung cancer chemoprevention trials (1–3) were initiated on the premise that β-carotene had potential as a chemopreventive agent (4). Unexpectedly, two of the trials reported a higher risk of lung cancer in subjects randomly assigned to receive β-carotene. The Finnish Alpha-Tocopherol, Beta-Carotene (ATBC) Study found that β-carotene (20 mg/day) increased the risk of lung cancer 18% compared with placebo (2), and the American Beta-Carotene and Retinol Efficacy Trial (CARET) reported that β-carotene (30 mg/day) administered with retinol (25 000 IU/day) increased the risk of lung cancer by 28% (3). The reasons for these findings remain unclear, but the results emphasize how little is known regarding the effects of β-carotene.

Both of the two trials reporting this adverse effect of β-carotene (2,3) had recruited subjects, primarily cigarette smokers, at high risk of lung cancer. By contrast, the one large trial (1) reporting no increased risk from β-carotene—the Physicians’ Health Study—had recruited healthy middle-aged physicians who had a low prevalence of smoking. The findings in these study populations suggest that cigarette smoking could play a role in possible carcinogenic effects of β-carotene supplementation. Moreover, in both the ATBC Study and the CARET (2,3), the increase in lung cancer incidence was seen only in subjects who drank alcohol (5,6), a pattern that has led to the additional hypothesis that alcohol intake may somehow modify the effect of β-carotene to increase lung cancer risk.

In these three large trials (1–3), β-carotene did not seem to increase the incidence of cancer generally (2,6–8), even among subjects who drank alcohol or smoked cigarettes (7,8). However, there has been little assessment of how alcohol intake or cigarette smoking might modify the effects of β-carotene on carcinogenesis outside the lung. To provide additional insight into this issue, we report here findings from a randomized, double-blind trial of antioxidants (including β-carotene) as preventive agents for recurrence of large-bowel adenomas.

Subjects and Methods

Details of the design and findings of the Antioxidant Polyp Prevention Study have been published previously (9). The study involved six clinical centers: Dartmouth–Hitchcock Medical Center, the Cleveland Clinic, the Lahey Clinic, the University of California at Los Angeles (later, the University of Southern California School of Medicine/Kaiser Hospital), the University of Iowa School of Medicine, and the University of Minnesota School of Public Health. Dartmouth Medical School was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the Coordinating Center, and the University of Minnesota was the
Pathology Center. Patients were recruited from gastroenterology and surgical practices associated with the clinical centers; within 3 months before study entry, each subject had at least one histologically confirmed adenoma removed from the large bowel during the period from December 1984 through June 1988, with the entire mucosa of the bowel visualized endoscopically and judged to be free of further polyps. A total of 981 eligible subjects were entered into a 3-month placebo run-in period, after which 864 subjects were randomly assigned in a 2×2 factorial design to placebo only (N = 214), β-carotene (25 mg/day; N = 217), vitamin C (ascorbic acid; 1000 mg/day) plus vitamin E (α-tocopherol; 400 mg/day) (N = 225), or β-carotene plus vitamin C plus vitamin E (N = 208) (Fig. 1). Subjects received endoscopy 1 year and 4 years after the qualifying endoscopy. A study pathologist reviewed all tissue removed from the bowel. The treatment phase of the study ended in December 1992.

At study entry, subjects completed a questionnaire regarding medical history and lifestyle habits (including cigarette smoking) and filled out a validated food-frequency questionnaire (10) that included questions regarding usual intake of beer, wine, and spirits. Serum β-carotene levels were determined by a high-performance liquid chromatographic assay (9). Details of the design and findings of the study have been published previously (9): neither β-carotene nor vitamins C and E affected overall adenoma recurrence. Of the 751 subjects who completed both follow-up examinations, 735 provided baseline smoking data, and 707 provided baseline data regarding both smoking and alcohol intake; these individuals form the basis of this report.

**Statistical Analysis**

The analyses focused on any adenomas found during the main risk period of the trial, which was the interval beginning immediately after the year 1 colonoscopy up to and including the year 4 examination. To assess later stages of carcinogenesis, we also considered “advanced lesions”—adenomas 1 cm or larger in diameter or those judged by the pathologist to be tubulovillous (25%–74% villous component) or villous (≥75% villous component) or to contain advanced dysplasia or invasive cancer. These tumors constitute 18% of the neoplasms diagnosed in study subjects.

We categorized smoking status as current smoker versus never or former smoker at baseline and alcohol intake as non-drinker, light drinker (≤1 drink/day, on average), or moderate/heavy drinker (>1 drink/day, on average). In dietary analyses, we analyzed the logarithm of baseline caloric intake, the residuals of the regression of log baseline dietary β-carotene intake on log caloric intake, and the residuals of the regression of log baseline fat intake on log caloric intake.

Crude and adjusted risk ratios (RRs) were used to assess β-carotene effects within strata of smoking and alcohol use. For multivariate analysis of all adenomas, we used generalized linear models (11) fit with a log link function to compute RRs. Estimates were adjusted for allocation to vitamins C and E and, in multivariate analyses, also for age, sex, study center, and length of follow-up. In some analyses, we also adjusted for dietary intake of calories, fat, and β-carotene. Interactions among cigarette smoking, alcohol intake, and β-carotene supplementation were assessed using product interaction terms and Wald tests. In an adjusted model that included β-carotene interactions with smoking and alcohol intake (any versus none) as well as the three-way interaction of these factors, the P values for the interactions were .008, .022, and .059, respectively. These terms were then included in a model that was used to estimate β-carotene effects within strata of smoking and alcohol intake, and these RRs were compared between strata using Wald tests. We assessed the effect of β-carotene on the multiplicity of adenomas.

---

**Fig. 1.** Consort diagram for subjects of the Antioxidant Polyp Prevention Trial. * Analysis of dietary beta carotene intake includes 373 placebo subjects. Analysis of serum levels includes 370 placebo subjects.
by using Poisson regression (11) to compute ratios of the numbers of adenomas in the β-carotene and placebo groups; the analysis strategy, independent variables, and adjustment factors were as described above. Generalized linear model analysis was similarly used to assess the impact of smoking and alcohol intake on associations of adenoma risk with baseline serum β-carotene and baseline dietary β-carotene intake. Computations were performed using STATA version 7 (Stata Corporation, College Station, TX). All statistical tests were two-sided, with P values <.05 considered statistically significant.

RESULTS

Overall, approximately 19% of the study subjects were current smokers at study entry, 71% reported some use of alcohol during the year before enrollment, and 26% reported that they had more than one alcoholic drink per day, on average (Table 1). The mean estimated dietary β-carotene intake at study entry was 4.7 mg, and the serum β-carotene level averaged 217.6 μg/L. Overall, β-carotene supplementation did not affect adenoma occurrence (adjusted RR = 1.01, 95% CI = 0.85 to 1.20) (9). Smoking showed no association with adenoma risk, but moderate to high alcohol intake increased risk (12).

The effect of β-carotene supplementation on the risk of adenoma recurrence varied substantially according to smoking habits and alcohol intake. Among subjects who reported that they neither smoked nor drank alcohol, β-carotene conferred a substantial protective effect: the adjusted RR for one or more adenomas was 0.56 (95% CI = 0.35 to 0.89) (Table 2). By contrast, among subjects who smoked cigarettes or drank alcohol, β-carotene supplementation conferred statistically nonsignificant increases in the risk of adenoma recurrence. These relative risks were statistically significantly different from the effects in subjects who did not smoke or drink alcohol (Table 2).

Among subjects who smoked cigarettes and also drank more than one alcoholic-containing drink per day, on average, β-carotene had a particularly strong adverse effect on adenoma recurrence (multivariate RR per 200 μg/L = 0.94, 95% CI = 0.79 to 1.11). For subjects in the placebo group who neither smoked nor drank alcohol, the adjusted RR was 0.72 (95% CI = 0.47 to 1.09); among those who both smoked and drank alcohol, the multivariate RR was 1.36 (95% CI = 0.67 to 2.76; P = .15 compared with that for RR for nonsmokers/nondrinkers). Baseline dietary β-carotene intake among subjects in the placebo group—as assessed by a food-frequency questionnaire—did not show substantial differences in RRs across smoking and alcohol groups (data not shown).

DISCUSSION

In this randomized, placebo-controlled clinical trial, we found that cigarette smoking and alcohol intake altered the effect of β-carotene supplementation on the risk of colorectal adenoma recurrence. Supplementation was beneficial among subjects who did not drink or smoke but, if anything, increased risk among those who drank alcohol and/or smoked.

There are substantial data indicating that β-carotene supplementation may increase the risk of lung cancer. In addition to the earlier trials that reported statistically significant RRs of 1.18 (95% CI = 1.03 to 1.36) and 1.28 (95% CI = 1.04 to 1.57) (5,6), two more recent trials (13,14) have reported statistically nonsignificantly increased lung cancer relative risks of approximately 1.4 or 1.5. One of these trials (14) was conducted among

Table 1. Characteristics of study participants included in the smoking/alcohol analyses*

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>β-Carotene</th>
<th>All subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>373</td>
<td>334</td>
<td>707</td>
</tr>
<tr>
<td>Mean age, y (SD)</td>
<td>61.5 (8.1)</td>
<td>60.8 (8.5)</td>
<td>61.1 (8.3)</td>
</tr>
<tr>
<td>No. of males (%)</td>
<td>301 (80.7)</td>
<td>253 (75.8)</td>
<td>554 (78.4)</td>
</tr>
<tr>
<td>Mean No. of lifetime adenomas (SD)</td>
<td>2.4 (2.2)</td>
<td>2.8 (4.0)</td>
<td>2.5 (3.2)</td>
</tr>
<tr>
<td>Smoking status, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>122 (32.7)</td>
<td>117 (35.0)</td>
<td>239 (33.8)</td>
</tr>
<tr>
<td>Former smokers</td>
<td>186 (49.9)</td>
<td>151 (45.2)</td>
<td>337 (47.7)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>65 (17.4)</td>
<td>66 (19.8)</td>
<td>131 (18.5)</td>
</tr>
<tr>
<td>Intake of alcoholic beverages, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondrinkers</td>
<td>103 (27.6)</td>
<td>99 (29.6)</td>
<td>202 (28.6)</td>
</tr>
<tr>
<td>&lt;1 drink/day</td>
<td>172 (46.1)</td>
<td>148 (44.3)</td>
<td>320 (45.3)</td>
</tr>
<tr>
<td>&gt;1 drink/day</td>
<td>98 (26.3)</td>
<td>87 (26.1)</td>
<td>185 (26.2)</td>
</tr>
<tr>
<td>Mean dietary β-carotene intake at study entry, mg/day (SD)†</td>
<td>4.7 (4.7)</td>
<td>4.6 (4.2)</td>
<td>4.7 (4.5)</td>
</tr>
<tr>
<td>Mean serum β-carotene level at study entry, μg/L (SD)†</td>
<td>217.0 (180.3)</td>
<td>218.2 (165.7)</td>
<td>217.6 (173.5)</td>
</tr>
</tbody>
</table>

*707 subjects in analyses presented here.
†Mean dietary β-carotene intake was determined from a food-frequency questionnaire, and mean serum β-carotene levels were assayed by high-performance liquid chromatography (9).
patients with head and neck cancer who, similar to the subjects in the ATBC Study and the CARET (5,6), were predominantly current and former cigarette smokers. Subjects in the other investigation—the Women’s Health Study (13)—were female health professionals who had a relatively low prevalence of smoking.

In the ATBC Study, the RR for lung cancer among the β-carotene group was 0.93 (95% CI = 0.65 to 1.33) among nondrinkers but was 1.35 (95% CI = 1.01 to 1.81) among those who reported consuming one or more drinks per day, on average (5). In the CARET, there was a similar interaction, with RRs of 1.07 (95% CI = 0.76 to 1.51) among nondrinkers and 1.99 (95% CI = 1.28 to 3.09) among β-carotene-supplemented subjects in the highest quartile of alcohol intake (6). In none of the previous trials did β-carotene supplementation seem to lead to an increased risk of colorectal cancer, even among subjects who reported drinking alcohol (5,7). In the Physicians’ Health Study, there were some suggestions that β-carotene supplementation could decrease colorectal cancer incidence among individuals who drink alcohol daily (7). In the ATBC Study, the effects of β-carotene supplementation on colorectal cancer risk did not seem to vary according to alcohol use (8).

The reasons for the differences between these findings regarding colorectal neoplasia and our results are not clear. Relatively few colorectal cancers were observed in those studies, so chance is clearly one possible explanation. An important additional possibility is that the interactions we found pertain to colorectal adenoma recurrence but not to the development of colorectal cancer itself. Arguing against this possibility, however, is our finding that advanced adenomas showed the same interaction pattern as all adenomas. This pattern suggests that the interactions are not limited to very early lesions.

In an earlier skin cancer prevention trial (15), we found that cigarette smoking modified the effect of β-carotene (50 mg/day) supplementation on skin cancer recurrence. Among nonsmokers, the adjusted RR was 0.97 (95% CI = 0.82 to 1.15), whereas among current smokers, the adjusted RR was 1.44 (95% CI = 0.99 to 2.09; P = .04 for interaction). These findings, together with the results presented here, suggest that the tendency for cigarette smoking and alcohol use to modify the effects of β-carotene supplementation on carcinogenesis extend beyond the lung and the particular characteristics of the subjects in the ATBC Study and the CARET (5,6). Our results also suggest that these effects are not limited to a particular form of β-carotene supplementation. Although these other two trials used β-carotene beadlets (which tend to produce relatively high increases in blood levels), our studies and two more recent trials (13,14) used capsules with powdered β-carotene (which, by comparison, tend to produce smaller increases in blood levels).

The effects of β-carotene on carcinogenesis are not well understood. Originally thought to be antineoplastic, partly because of its presumed antioxidant potency (4), β-carotene is an antioxidant at low oxygen pressures, but at ambient or high oxygen pressures, it can display prooxidant properties (16). This characteristic may be a result of the formation of oxidized metabolites that can enhance lipid peroxidation and increase formation of DNA adducts (17–19). High oxygen tensions are not achieved in the bowel (20), but cigarette smoking seems to increase the production of oxidized β-carotene metabolites (21–23). If this occurs in the colorectal mucosa, it may provide a physiologic basis for our observed interaction between smoking and β-carotene.

Other possibly procarcinogenic effects of β-carotene have also been proposed. High doses of β-carotene, but not physiologic doses, may decrease tissue concentrations of retinoic acid, decrease expression of the retinoic acid receptor β, and increase AP-1 expression, all of which would be expected to enhance carcinogenesis (21,24). It is conceivable that these effects might differ between individuals who drink alcohol or smoke cigarettes and those who do not, but we are unaware of evidence supporting that possibility.

Our data suggest that ethanol intake may counter a protective effect of β-carotene on neoplasia in the bowel. Unfortunately, the effects of alcohol intake on the process of carcinogenesis are not well understood. Ethanol is not carcinogenic or mutagenic in animals, but intake of alcoholic beverages in humans is clearly associated with an increased risk of cancers at several anatomic sites (25–27). Several mechanisms for such a neoplastic effect have been proposed, including induction of phase I enzymes, inhibition of phase II enzymes, disturbances of retinol metabolism, interference with DNA repair, and interference with folate metabolism (25,28–32). Like β-carotene, alcohol may increase expression of AP-1 and decrease expression of the retinoic acid receptor β (33–38). Acetaldehyde, the major metabolite of ethanol, is a known animal carcinogen (25,29) and may increase cell proliferation in the distal large bowel (39). In rodent studies (40), acetaldehyde produced from alcohol by microbes may promote folate deficiency in the bowel mucosa, despite normal serum

### Table 2. β-Carotene risk ratios for adenoma recurrence among subjects of the Antioxidant Polyp Prevention Trial

<table>
<thead>
<tr>
<th>Smoking/drinking status</th>
<th>Placebo</th>
<th>β-Carotene</th>
<th>Adjusted</th>
<th>P†</th>
<th>Multivariate</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmoker‡, nondrinker</td>
<td>37/88 (42.1)</td>
<td>18/81 (22.2)</td>
<td>0.53 (0.33 to 0.84)</td>
<td>Referent</td>
<td>0.56 (0.35 to 0.89)</td>
<td>Referent</td>
</tr>
<tr>
<td>Smoker, nondrinker</td>
<td>5/15 (33.3)</td>
<td>8/18 (44.4)</td>
<td>1.31 (0.67 to 2.55)</td>
<td>.019</td>
<td>1.36 (0.70 to 2.62)</td>
<td>.022</td>
</tr>
<tr>
<td>Drink, nonsmoker‡</td>
<td>82/229 (37.3)</td>
<td>77/187 (41.2)</td>
<td>1.11 (0.87 to 1.41)</td>
<td>.005</td>
<td>1.13 (0.89 to 1.45)</td>
<td>.008</td>
</tr>
<tr>
<td>≤1 drink/day</td>
<td>47/147 (32.0)</td>
<td>40/116 (34.5)</td>
<td>1.07 (0.76 to 1.49)</td>
<td>.015</td>
<td>1.10 (0.79 to 1.54)</td>
<td>.017</td>
</tr>
<tr>
<td>&gt;1 drink/day</td>
<td>35/73 (48.0)</td>
<td>37/71 (52.1)</td>
<td>1.13 (0.82 to 1.56)</td>
<td>.008</td>
<td>1.09 (0.80 to 1.49)</td>
<td>.018</td>
</tr>
<tr>
<td>Smoker, drinker</td>
<td>15/50 (30.0)</td>
<td>18/48 (37.5)</td>
<td>1.27 (0.75 to 2.13)</td>
<td>.017</td>
<td>1.33 (0.81 to 2.17)</td>
<td>.015</td>
</tr>
<tr>
<td>≤1 drink/day</td>
<td>7/25 (28.0)</td>
<td>7/32 (21.9)</td>
<td>0.89 (0.41 to 1.93)</td>
<td>.26</td>
<td>0.89 (0.42 to 1.89)</td>
<td>.30</td>
</tr>
<tr>
<td>&gt;1 drink/day</td>
<td>8/25 (32.0)</td>
<td>11/16 (68.8)</td>
<td>1.98 (1.19 to 3.30)</td>
<td>&lt;.001</td>
<td>2.07 (1.39 to 3.08)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Risk ratios and 95% confidence intervals (CIs) were adjusted for supplementation with vitamins C and E (adjusted) or were adjusted for age, sex, study center, interval between examinations, and supplementation with vitamins C and E (multivariate).
†P for difference in β-carotene risk ratio from that for nonsmokers/nondrinkers.
‡Never or former smoker.
folic acid levels. However, specific interactions with β-carotene in these contexts have not been proposed.

The pharmacologic interactions between β-carotene and ethanol are not entirely clear. In cross-sectional studies (41,42) and some trials of β-carotene supplementation (43,44), alcohol intake has been associated with reduced serum β-carotene levels. However, in a feeding study that carefully controlled carotenoid intake, alcohol administration was associated with increased β-carotene levels (45). At least in animal models, β-carotene may increase the liver damage caused by alcohol intake, which in turn could interfere with the conversion of β-carotene to retinol (30). Both alcohol and β-carotene induce cytochrome P450 2E1 (46,47) but, to our knowledge, there has been no investigation of whether a pharmacologic interaction occurs. The relationship of all these effects with carcinogenesis in the bowel is not clear.

There are several possible reasons for the differences between our findings for β-carotene supplementation and dietary β-carotene intake. The findings for supplementation may not be pertinent for the lower intake usually obtained from diet alone. However, data from our study argue against this possibility. Indeed, we found similar patterns when we considered effects of β-carotene supplementation on adenoma recurrence and associations with serum levels in the placebo group, which reflect dietary intake. More likely, the measurement error inherent in dietary assessment and the nonrandomized assessment of β-carotene intake probably obscured the interactions.

Although our analysis has the advantage of randomized β-carotene supplementation, alcohol intake and cigarette smoking were habits taken up by the subjects themselves. Consequently these exposures bring with them the limitations of most observational analyses, including the potential for measurement error and association with other unknown lifestyle factors. The effect of these possible problems on our findings is not clear.

Our findings suggest that β-carotene may have antineoplastic effects in some individuals, in particular, among those who abstain from using alcohol and tobacco. Yet, in some circumstances, β-carotene seems to be proneoplastic, for example, among individuals who drink alcohol or smoke cigarettes. These results suggest that caution must be applied in choosing interventions for large-scale use in well people, particularly when the mechanisms of action and possible interactions with lifestyle factors are not well understood.

REFERENCES


Homann N, Tillonen J, Salaspuro M. Microbiially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. Int J Cancer 2000;86:169–73.


NOTES

Supported by Public Health Service grants CA37287 and CA23108 (to J. A. Baron) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

We thank the many investigators who supported the study and the patients who took part.

Manuscript received August 27, 2002; revised March 5, 2003; accepted March 17, 2003.