β-Carotene Treatment of Cervical Intraepithelial Neoplasia: A Phase II Study[1]

Alberto Manetta,[2] Toni Schubbert, Julia Chapman, Michael J. Schell, Yei-Mei Peng, Shu Yuan Liao, and Frank J. Meyskens, Jr.

Division of Gynecological Oncology [A. M., T. S., J. C.], Department of Medicine, Hematology/Oncology [M. J. S.], Department of Medicine, Division of Epidemiology [S. Y. L.], and Clinical Cancer Center [M. J. S., F. J. M.], University of California, Irvine, Irvine, California 92668, and Arizona Cancer Center, Tucson, Arizona 85724 [Y-M. P.]

Abstract

The use of Papanicolaou smears for cervical cancer screening has led to an increased detection of preinvasive conditions of the cervix, cervical intraepithelial neoplasia (CIN). Epidemiological studies have shown an association between low levels of dietary β-carotene and CIN. In this Phase II study, we have explored the effect of p.o. β-carotene administration on CIN I and II. Patients with documented CIN I or II were treated with 30 mg daily of β-carotene for 6 months. Response rates were determined at 0, 3, 6, and 12 months with cytology, colposcopy, and/or biopsies. Levels of β-carotene and vitamin E were determined at the same time intervals in vaginal mucosa cells and serum. Response rates were 18 of 30 (60%), 21 of 30 (70%), and 10 of 30 (33%) at 3, 6, and 12 months, respectively. Significant changes occurred in the serum β-carotene levels over time. Median levels over 2200 mg/ml were found at 3 and 6 months *versus* a baseline median level of 111 ($P < 0.0001$). Significant increases were also noted in the β-carotene levels of the vaginal mucosa compared to baseline ($P = 0.01$) and a significant correlation was noted between serum and vaginal β-carotene levels as well ($P < 0.0001$). This study indicates that a large percentage of patients with CIN I and II will respond clinically to p.o. β-carotene supplementation. There is a positive relationship between serum and tissue levels of β-carotene which suggests that serum levels can be used for monitoring purposes. Because of these encouraging results, prospective randomized studies are ongoing comparing the efficacy of β-carotene against an untreated control arm.

Introduction

Carcinoma of the cervix is one of the most common and deadly malignancies in the developing world. In some countries, it is the leading cause of death for women between the ages of 30 and 50. In the United States, the incidence and mortality rate of cervix cancer has declined over the last few decades with approximately 13,000 women developing the disease and 6,000 dying annually. This decreasing incidence is attributed to the effectiveness of screening programs. Because of a well-established premalignant phase, ease of use of Pap smears, low cost, and a high degree of acceptability, screening programs have been extremely successful in decreasing the incidence of invasive cervix cancer. However, the success of this program has led to a significant increase in the detection of CIN.[3] Because of the easy accessibility for biopsy and inspection, cancer of the cervix is an ideal tumor for the study of chemopreventive agents.

An association has been found between the incidence of CIN and dietary levels of vitamin A and/or β-carotene (1–4). The risk of cervical cancer was also found to be higher among women with lower serum levels of total carotenoids (4). β-carotene has been used with success in the management of premalignant lesions such as p.o. leukoplakia (5, 6).

Previous clinical trials have demonstrated the potential use of topical β-*trans*-retinoic acid in the management of CIN (7, 8). However, local toxicity was commonly experienced. In contrast, β-carotene has been found to be relatively nontoxic to humans; at high doses, a slight yellowing of the skin may occur. Because of this lack of side effects, β-carotene has received attention as a chemopreventive agent. In this Phase II trial, we have obtained preliminary data on the effect of β-carotene on CIN. In addition, we investigated the relationship between the serum and tissue levels of micronutrients and their response to p.o. β-carotene supplementation.

Materials and Methods

This nonrandomized Phase II study was intended to determine the activity and side effects of β-carotene in the management of patients with CIN I and II.

Eligibility criteria for study entry were the presence of an intraepithelial lesion recognizable using colposcopy and documented by biopsy as CIN I or II. Eligible patients had residual lesion or lesions remaining on the cervix following the biopsy. Patients with positive endocervical curettetage on previous or concomitant examinations were not eligible for this protocol. Also ineligible were patients who had been exposed *in utero* to diethylstilbestrol. All patients underwent initial cervical cytology and colposcopy with mapping of the cervical lesion on appropriate forms. All colposcopies were performed either by a

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[1] Supported in part by Hoffman-LaRoche.
[2] To whom requests for reprints should be addressed, at Department Obstetrics and Gynecology, University of California, Irvine Medical Center, 101 The City Drive, Building 23, Orange, CA 92668. Phone: (714) 456-6570; Fax: (714) 456-6463.

[3] The abbreviation used is: CIN, cervical intraepithelial neoplasia.

gynecological oncologist or a gynecological oncology fellow. Standard criteria for evaluation of CIN were used by both investigators. Quadrant extension of the lesion was also noted. Colposcopically directed biopsies were performed in all patients. An informed consent approved by the Institutional Review Board at University of California, Irvine was signed by all patients.

Pathologists who were blinded as to the patients' participation in the study graded the biopsies as normal or CIN 1, 2, or 3.

Vaginal and serum levels of β-carotene, retinol, and vitamin E were determined at 0, 3, 6, and 12 months. Analysis was performed as described previously (9). Serum samples were obtained on 86 of 122 (70%) of the total patient visits. The majority of missing samples (23/36) occurred among the first 10 patients because of a technical failure. Cervical-vaginal cell samples were obtained from 16 patients in 46 of 52 (88%) visits during the second half of the study. A cytobrush was used to collect cells from the vaginal wall. The cells were stored and transported in saline solution at 4°C.

Patients received 30 mg/day of β-carotene for 6 months and were assessed for extent of disease at baseline and for response at 3, 6, 9, and 12 months. Pap smears were routinely performed at each follow-up visit. All patients had biopsies at baseline. At subsequent visits, biopsies were only performed if the colposcopy suggested progression of the dysplasia. If progression was confirmed, patients were then removed from the study and considered treatment failures. Patients with stable disease at 9 months were removed from study and treated conventionally. Patients who achieved a response were followed for an additional 6 months off therapy.

Patients were considered responders if the Pap smear and colposcopy reversed to normal. In cases with negative Pap smears but suspicious colposcopy, biopsies were taken to determine response. All patients with abnormal Pap smears or positive biopsies were considered to be nonresponders.

Statistical Methods. Comparison of response probability by disease stage or extent (quadrants involved) were made using the Fisher exact test. To avoid introducing a bias due to patient drop-off, patients who dropped off study were considered to be nonresponders at all subsequent times. The Kruskal-Wallis test was used to compare micronutrient levels with time. Correlations were assessed using the Spearman rank correlation method. The interquartile range gives the 25th and 75th percentile values for the variable being described, thus providing the limits for the central half of the data.

Cell counts from fresh cervical-vaginal cells were scored as <1, 1, 2, or 3 million cells. When the number of cells was reported as <1 million (n = 15), 0.7 million was used. Since the lowest observed β-carotene level was 0.10 ng/million cells, a value of 0.05 ng/million cells was used in the 14 instances when the level was not detectable. Formal comparison of the serum micronutrient and cellular β-carotene levels was done only in the patients for whom data both on (3 or 6 months) and off (0, 9, or 12 months) β-carotene supplementation was available using the Wilcoxon signed rank test. Whenever multiple levels were available for a patient, the values were averaged.

Results

Of the 30 patients enrolled in the trial, five patients were taken off the study due to either disease progression (two at 3 months and one at 6 months) or persistent disease at 9 months (n = 2). Three additional patients were removed from the study due to pregnancy (one at 3 months and 2 at 6 months). Five patients dropped off from the study because of relocation (one at 3 months and four at 6 months).

Table 1 Response and number of quadrants involved

Quadrant	Responders	
	6 mo	12 mo
1–2	19/22 (86%)	10/22 (45%)
3–4	2/8 (25%)	0/8 (0%)
	P = 0.003[a]	P = 0.03[a]

[a] Comparison of one to two *vs.* three to four quadrants using the two-sided Fisher exact test.

At 6 months, 21 patients (70%) were responders (95% confidence interval, 51–85%), whereas at 1 year 10 patients (33%) were responders (95% confidence interval, 17–53%). Of the 11 patients who were responders at 6 months but not at 1 year, 6 had recurrence of dysplasia and 5 were patients who dropped off the study. One of the patients who withdrew at 6 months had CIN II dysplasia at baseline but was biopsy negative at 6 months.

Patients were requested to report the number of pills not taken, every 3 months. Compliance was good with all patients able to take β-carotene as prescribed. This was further confirmed by significant elevation of β-carotene serum levels on all patients tested. No side effects were reported by any of the patients participating in the study. None of the patients who dropped off did so because of side effects. The severity of disease was not significantly associated with the probability of response: 14 of 18 (78%) patients with CIN I were responders at 6 months compared to 7 of 12 (58%) patients with CIN II (P = 0.42) and 6 of 18 (33%) patients with CIN I were responders at 12 months compared to 4 of 12 (33%) patients with CIN II (P = 1.0).

The likelihood of response did depend on disease extent as measured by the number of quadrants (quadrants 1–4) of the cervix involved with CIN. Nineteen of 22 patients (86%) with one to two quadrants involved had responded at 6 months *versus* 2 of 8 patients (25%) with three- to four-quadrant involvement (P = 0.003). Ten of 22 patients (45%) with one to two quadrants involved were responders at 12 months compared to 0 of 8 patients with three to four quadrants involved (P = 0.03; Table 1). Of the patients who dropped off the study at 3 and 6 months, two of two and one of six had disease involvement in three or four quadrants. Both tests remain statistically significant even if the patients who dropped off the study were excluded from the analysis.

Serum β-carotene levels were significantly higher when the patients were receiving β-carotene supplementation (P = 0.0001) using the Wilcoxon signed rank test for paired data. The median baseline serum β-carotene level was 111 ng/ml, with one half of the patients having values between 62 and 159 ng/ml (Table 2). This level increased to roughly 2300 at 3 and 6 months when the patients took β-carotene supplementation. The serum levels declined to 412 at 9 months and 243 at 12 months, values still substantially higher than baseline levels (P = 0.003 and P = 0.004) Vitamin E levels were also higher at 3 and 6 months (P = 0.0004), but no difference in retinol levels was noted (P = 0.91; Table 2). Strong correlations were noted between baseline and 12 months for β-carotene, retinol, and vitamin E (r = 0.87, 0.90, and 0.82, respectively; n = 10). Correlation between the baseline serum β-carotene levels and after 6 months is much lower (r = 0.39). There was no significant relationship between the serum level of β-carotene and response.

Table 2 Serum micronutrient levels

Time (mo)	*n*	Md (ng/ml)	Interquartile range	Range
β-Carotene				
0	18	111	62–159	22–324
3	21	2254	1198–3252	94–4653
6	20	2270	1718–3344	58–4078
9	13	412	216–478	52–1222
12	12	243	146–360	71–391
Retinol				
0	18	400	353–531	176–693
3	21	450	393–595	229–710
6	20	485	408–540	198–708
9	14	505	416–587	282–722
12	13	445	353–505	264–723
Vitamin E				
0	18	8979	6993–10111	3930–13324
3	21	9327	8523–11681	5087–18545
6	20	9315	8630–10903	4920–18305
9	14	8787	8050–9889	5216–14232
12	13	8771	7641–10277	5165–12651

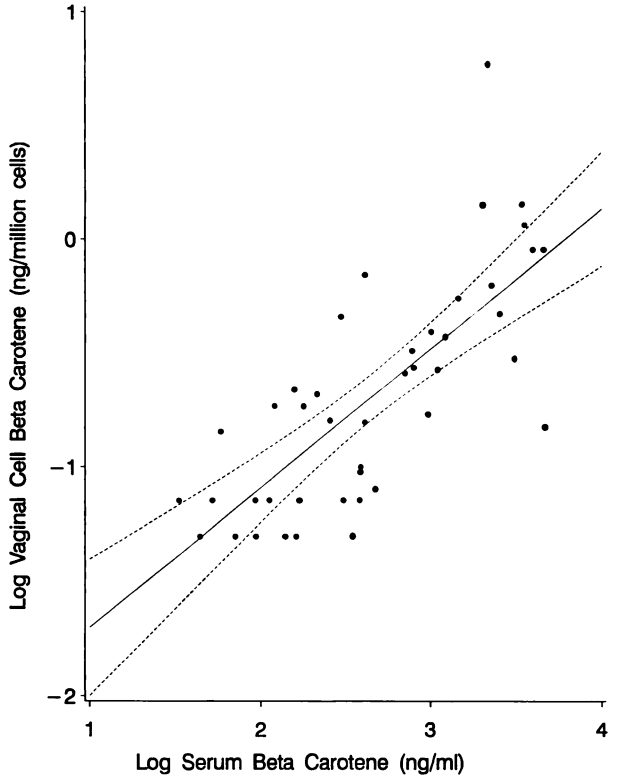

Fig. 1. Plot of serum *versus* vaginal β-carotene (n = 43). ——, linear regression fit; - - - -, 95% confidence interval.

Fig. 1 shows the relationship between the logarithm of serum and vaginal β-carotene levels for 43 samples (r = 0.75). Eleven patients had β-carotene levels measured on cervical-vaginal cells both on (3 or 6 months) and off (0, 9, or 12 months) β-carotene supplementation (Fig. 2). The β-carotene level, which rose in 10 of the 11 patients, showed a statistically significant increase when patients were on supplementation (P = 0.007). The lone patient with a reduced vaginal β-carotene level also had a lower serum β-carotene at 3 and 6 months

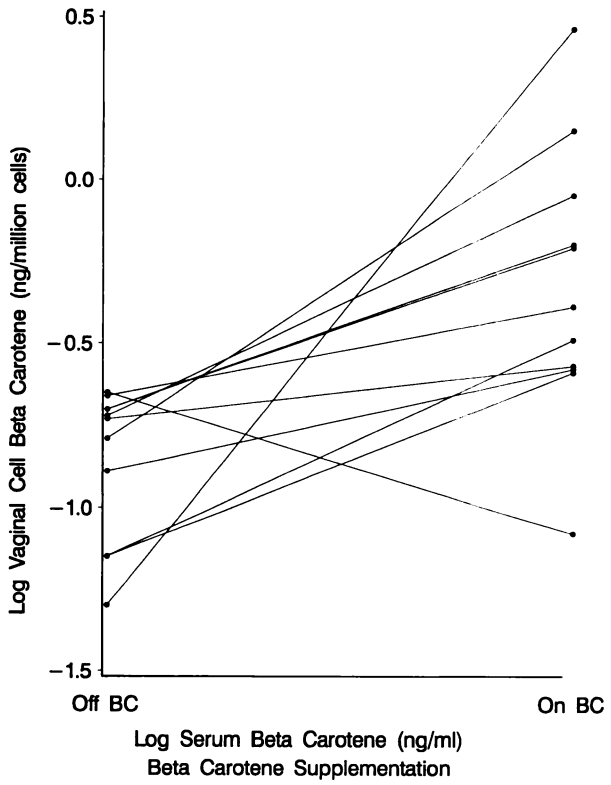

Fig. 2. Plot of vaginal β-carotene by β-carotene supplementation in 11 patients who were studied both on and off β-carotene. *Lines* join data from the same patient.

compared to baseline levels. (In one patient, no comparison of serum β-carotene was possible and in the remaining nine patients the level rose while on supplementation.) Thus, it is likely that this patient did not take β-carotene supplementation as instructed.

Discussion

These results suggest that a significant percentage of patients with CIN will respond to β-carotene supplement at a dose of 30 mg daily. Seventy percent of the patients (21/30) at 6 months and 30% (10/30) at 12 months exhibited a response of their CIN to β-carotene. Five patients who were complete responders dropped out of the study at 6 months due to pregnancy or other reasons, which impacted the total percentage of responders at 12 months. Eighteen patients were considered responders at 3 months. This increased to 21 at 6 months. Only one patient who was a responder at 3 months recurred between 3 and 6 months. However, six patients recurred between 6 and 12 months. (After β-carotene supplementation was discontinued.) Nevertheless, 10 of 16 patients who were responders at 6 months and completed the protocol continued to respond after 12 months. Thus, we might anticipate that three of the five responders who dropped off at 6 months would have been responders at 12 months had they remained on study. Hence, we estimate the true response rate at 12 months to be about 43% (13/30). In contrast, deVet *et al.* (11) were not able to show a beneficial effect of β-carotene on the regression rate of cervical dysplasia. However, in their study, patients were treated with only 10 mg of β-carotene daily for 3 months.

We were also able to demonstrate that increased intake of β-carotene translated into significant increases in serum and vaginal cell levels.

In this study, we also found a difference in the response rate to β-carotene according to the number of quadrants of the cervix involved. Patients with small lesions occupying only one and two quadrants responded better than patients with larger lesions. This indicates that in future studies surface extension of the lesion in addition to the degree of dysplasia should be considered as a stratification factor at the time of study entry. The end points selected in this study closely adhere to the present standard of care. Patients with abnormal Pap smears and/or abnormal colposcopies underwent cervical biopsies. Patients with normal Pap smears and colposcopies did not undergo biopsies but continued to be maintained with cytology and colposcopy. This could be viewed as a limitation of this study since not all patients were biopsied. However, it is the unusual patient that will show dysplasia on random cervical biopsies in the absence of abnormalities in the Pap smear or colposcopy.

Although the outcome of this Phase II study is encouraging, the interpretation is difficult due to the variable rate (0–60%) of the spontaneous regression for CIN seen in different populations (12, 13). In general, the lowest report of regression rates were from studies where the original diagnosis, as well as the follow-up, has been performed using cytology and colposcopy exclusive of cervical biopsies in contrast to higher regression rates reported by investigators making the diagnosis and following up patients with cervical biopsies. There is the possibility that single-punch biopsy not only removes the lesion, but also beneficially influences the remaining epithelium (14). Because of the variable regression rate for low-grade CIN, a prospective randomized study will need to be completed to determine whether β-carotene has efficacy in the management of CIN, a trial currently ongoing at our institution.

References

1. Wylie-Rosett, J. A., Romney, S. L., Slage, S., Wassentheil-Smoller, S., Miller, G. L., Palan, P. R., Lucido, D. L., and Duttagupta, C. Influence of vitamin A on cervical dysplasia and carcinoma *in situ*. Nutr. Cancer, *6:* 47–49, 1984.

2. Herrero, R., Potischman, N., Brinton, L. A., Reeves, W. C., Brenes, M. M., Tenorio, F., deBritton, R. C., and Gaitan, E. A case-control study of nutrient status and invasive cervical cancer. I. Dietary Indicators. Am. J. Epidemiol., *134:* 1335–1346, 1991.

3. Potischman, N., Herrero, R., Brinton, L. A., Reeves, W. C., Staccwicz-Sapuntzakis, M., Jones, C. J., Brenes, M. M., Tenorio, F., deBritton, R. C., and Gaitan, E. A case-control study of nutrient status and invasive cervical cancer. II. Serologic indicators. Am. J. Epidemiol., *134:* 1347–1355, 1991.

4. Batieha, A. M., Armenian, H. K., Norkus, E. P., Morris, J. S., Spate, V. E., and Comstock, G. W. Serum micronutrients and the subsequent risk of cervical cancer in a population-based nested case-control study. Cancer Epidemiol., Biomarkers & Prev., *2:* 335–339, 1993.

5. Koch, H. F. Biochemical treatment of precancerous oral lesions: the effectiveness of various analogues of retinoic acid. J. Maxillofac. Surg., *6:* 59–63, 1978.

6. Garewal, H. A. S., Meyskens, F., Jr., Killen, D., Reeves, D., Kiersch, T. A., Elletson, H., Strosberg, A., King, D., and Steinbraun, K. Response of oral leukoplakia to β-carotene. J. Clin. Oncol., *8:* 1715–1720, 1990.

7. Meyskens, F. L., Jr., Graham, V., Chvapil, M., Dorr, R. T., Alberts, D. S., and Surwit, E. A. A phase I trial of β-all-*trans*-retinoic acid delivered via a collagen sponge and a cervical cap for mild or moderate intraepithelial cervical neoplasia. J. Natl. Cancer Inst., *71:* 921–925, 1983.

8. Surwit, E. A., Gramah, V., Droegemueller, W., Alberts, D., Chvapil, M., Dorr, R. T., Davis, J. R., and Meyskens, F. L. Evaluation of topically applied *trans*-retinoic acid in the treatment of cervical intraepithelial lesions. Am. J. Obstet. Gynecol., *143:* 821–823, 1982.

9. Peng, Y. S., Peng, Y. M., McGee, D. L., and Alberts, D. S. Carotenoids, tocopherols and retinoids in human buccal mucosal cells: intra-and interindividual variability and storage stability. Am. J. Clin. Nutr., *59:* 636–643, 1994.

10. Palan, P. R., Mikhail, M. S., Basu, J., and Romney, S. L. β carotene levels in exfoliated cervicovaginal epithelial cells in cervical intraepithelial neoplasia and cervical cancer. J. Obstet. Gynecol., *167:* 1899–1903, 1992.

11. DeVet, H. C. W., Knipschild, P. G., Willebrand, D., *et al.* The effect of β-carotene on the regression and progression of cervical dysplasia: a clinical experiment. J. Clin. Epidemiol., *44:* 273–283, 1991.

12. Scott, R. B., and Ballard, L. A. Problems of cervical biopsies. Ann. NY Acad. Sci., *1997:* 767–781, 1962.

13. Lerch, V., Okagaki, T., Austin, J. H., Kevorkian, A. Y., and Young, P. A. Cytologic findings in the progression of anaplasia (dysplasia) to carcinoma *in situ*: a progress report. Acta Cytol., *7:* 183–186, 1963.

14. Richart, R. M., and Barron, B. A. A follow-up study of patients with cervical dysplasia. Am. J. Obstet. Gynecol., *105:* 383–393, 1969.