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# Prevention of cervical intraepithelial neoplasia and cervical cancer[1–3]

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**ABSTRACT** We review the current status of prevention trials in the management of cervical intraepithelial neoplasia (CIN) and cervical cancer. Two large, randomized controlled trials have shown that folic acid is inactive in reversing low to moderate grade CIN. A large randomized trial of locally applied β-*trans* retinoic acid showed that the agent was effective in reversing moderate but not severe CIN. Results from a pilot trial involving 30 patients with CIN I (mild dysplasia) and CIN II (moderate dysplasia) indicate that β-carotene can suppress CIN; a large ongoing randomized trial will answer the question more definitively. *Am J Clin Nutr* 1995;62(suppl):1417S–9S.

**KEY WORDS** Cervix cancer, chemoprevention trial, β-carotene, folic acid, retinoic acid

## BACKGROUND

A considerable amount of data suggests that nutrients may play a role in the natural history of cervical intraepithelial neoplasia (CIN) and cervical carcinoma (1–10) (**Table 1**). Folic acid is critical to DNA synthesis and methylation, and deficiency in folic acid or abnormalities in its metabolism lead to megaloblastic changes in numerous tissues, including the cervix. Megaloblastic features observed by Papanicolaou smears and related to oral contraceptive use can be reversed by folic acid supplementation (11). Additional studies by Butterworth et al (12) showed that folic acid supplementation in women who used oral contraceptives could improve CIN I and CIN II, at least over the short term (3 mo) of the trial (12). These authors also noted a statistically lower mean red blood cell folate concentration in oral contraceptive users compared with nonusers, which was particularly marked in patients with CIN. Epidemiologic investigations of the role of folic acid in CIN and cervical cancer also consistently support a protective role, although not a strong one (6). These preliminary studies were sufficiently encouraging that definitive phase III trials were launched.

The status of vitamins A and C and β-carotene nutriture seems to have a substantial role in the development of CIN and cervical cancer as determined by various epidemiologic studies. Persons who have low serum concentrations of these nutrients or low dietary intake are at increased risk; the data are strongest for ascorbic acid and β-carotene (1, 3, 9). The association of CIN with cigarette smoking has also extended these observations, because β-carotene is known from a large number of studies to be protective against lung cancer. Subsequently, this nutrient was shown to be effective against smoking-related CIN and cervical cancer (4, 13). Whether this protective effect occurs through an antioxidant-mediated mechanism is unknown but seems reasonable. The carotenoid may affect its response directly or by conversion to vitamin A and subsequently β-*trans* retinoic acid, which is generally thought to represent the end ligand in affecting cellular change (14). In general, vitamin A and its natural and synthetic derivatives (retinoids) modulate the growth of epithelial cells—slowing growth and enhancing maturation (15, 16). Retinoic acid was also shown to influence the maturation state of keratinocytes infected with human papilloma virus (17), an important observation because these viruses appear to be important in the etiology of cervical cancer. The amassing evidence suggests that low vitamin nutriture, cigarette smoking, and human papilloma virus infection combine to contribute to the evolution of CIN.

## CLINICAL TRIALS

Two large, placebo-controlled, randomized trials using folic acid in the treatment arm have convincingly shown that increased regression of CIN I and II over a 3–6-mo period does not occur (18, 19). Neither trial addressed whether folic acid deficiency corrected before the development of histologic changes would alter the eventual outcome because both trials enrolled subjects who already had CIN. To answer this critical issue, future trials will need to enlist folic acid–deficient women without CIN; for example, women with normal Papanicolaou smears in a sexually transmitted disease clinic might be a good study population.

Over the past 15 y, we have conducted a series of pilot and phase I and II trials to determine whether β-*trans* retinoic acid applied topically to the cervix might reverse or suppress CIN (20–22). The phase II trial resulted in a 50% complete clinical response rate (21), which was sufficiently encouraging that a randomized phase III trial was undertaken; the key results from that study are summarized in **Table 2** (23).

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**TABLE 1**
Strength of evidence that candidate chemopreventives prevent cervical cancer[1]

| Compound | Type of evidence | | |
|---|---|---|---|
| | Metabolic or tissue culture | Epidemiologic | Clinical trial |
| Folic acid | ++ | ++ | Phase II positive, 1 phase III trial negative |
| Vitamin A | + | + | NA |
| β-Carotene | ++ | ++ | Phase II,[2] and Phase III[3] |
| Vitamin C | + | +++ | Phase III[3] |

[1] +, weak and/or few reports; +++, strong and/or many reports. NA, not available.
[2] Phase II trial completed; 60% positive response rate.
[3] Phase III trials in cervical intraepithelial neoplasia just started in 1994 at two different institutions, completion in 3–4 y.

This randomized phase III trial was designed to determine whether topically applied retinoic acid reversed moderate CIN II or severe CIN. Analyses were based on 301 women with CIN (151 women with moderate dysplasia and 150 women with severe dysplasia), evaluated by serial colposcopy, Papanicolaou cytology, and cervical biopsy. Cervical caps with sponges containing either 1.0 mL 0.372% β-*trans* retinoic acid or a placebo were inserted daily for 4 d when women entered the trial and for 2 d at months 3 and 6. Patients receiving treatment and those receiving placebo were similar with respect to age, ethnicity, birth control methods, histologic features of the endocervical biopsy and koilocytotic atypia, and percentage of involvement of the cervix at study. Treatment effects were compared using Fisher's exact test.

Retinoic acid increased the complete histologic regression rate of CIN II from 27% in the placebo group to 43% in the retinoic treatment group ($P = 0.041$). No treatment difference between the two arms was evident in the severe dysplasia group. More vaginal and vulvar side effects were seen in the patients receiving retinoic acid, but these were mild and reversible. These results were encouraging and suggested that this modality of management might be useful in some patients. The study also provided further support to the notion that chemoprevention of human cancer is feasible.

In addition to the substantial epidemiologic data that support a protective role of β-carotene against many cancers (particularly those associated with cigarette smoking), recent clinical studies have shown that β-carotene can affect marked regressions in some cases of oral leukoplakia (24). On the basis of these epidemiologic and clinical observations, we recently completed a phase II trial of oral β-carotene for CIN. Strict eligibility criteria were followed and required lesions completely visualized by colposcopy, biopsy-proven CIN I and II, measurable residual lesions present after biopsy, negative endocervical curettage, and no prior or concomitant malignancy or in utero exposure to diethylstilbestrol. Thirty patients were treated and received 30 mg β-carotene orally per day for up to 6 mo. Responders were required to have a colposcopy and a Papanicolaou smear with negative findings.

**TABLE 2**
Comparison of endpoint to entry biopsy in participant with cervical intraepithelial neoplasia (CIN)[1]

| Dysplasia and intervention | Regression[2] | Other[3] |
|---|---|---|
| Moderate | | |
| Retinoic acid | 32 (43%) | 43 |
| Placebo | 18 (27%) | 48 |
| Severe | | |
| Retinoic acid | 10 (25%) | 30 |
| Placebo | 16 (31%) | 35 |

[1] Reproduced from reference 22.
[2] Regression is complete biopsy response (or endocervical curettage if biopsy not available) at 15, 9, 12, 21, or 27 mo as defined in the text. $P = 0.041$ for moderate dysplasia and $P = 0.33$ for severe dysplasia.
[3] Other is no response or CIN upstaged.

**TABLE 3**
Number of patients evaluable and overall response rate in phase II trial of β-carotene in the management of patients with cervical intraepithelial neoplasia (CIN) I and II[1]

| | 3 mo | 6 mo | 12 mo |
|---|---|---|---|
| Response rate | 18/30 (60%) | 21/30 (70%) | 10/30 (33%) |

[1] After 3 mo patients were continued on β-carotene if they were stable or responding to the treatment. After 6 mo, β-carotene was stopped in all patients.

Based on these criteria > 70% of patients with CIN I or II had responded to oral β-carotene by 6 mo (**Table 3**). Subset analysis showed that the severity of disease (CIN I versus CIN II) did not predict response while extent (one to two quadrants versus three to four quadrants, $P = 0.002$ at 6 mo) did. Measurement of β-carotene in serum and shed cervicovaginal cells showed that a plateau was obtained after 3 mo of oral administration and that there was an excellent correlation ($R = 0.70$) between the two indexes. Interestingly, β-carotene did not produce serum suppression of either vitamin E or A at any point.

These results are encouraging and therefore a randomized phase III trial has been undertaken. Various biologic intermediate markers will be extensively measured.

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