Cervical Tissue and Plasma Concentrations of α-Carotene and β-Carotene in Women Are Correlated\textsuperscript{1,2,3}

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ABSTRACT Results from epidemiologic studies suggest that a carotenoid-rich diet may reduce risk for cervical cancer, possibly by inhibiting the progression of cervical intraepithelial neoplasia, a preneoplastic lesion of the cervical tissue. Laboratory studies suggest that the mechanism may be linked to the metabolism of carotenoids to retinoic acid or retinoic acid–like compounds, which has been hypothesized to occur in the cervical tissue. The purpose of this study was to demonstrate the presence of provitamin A carotenoids in biopsied samples of this peripheral tissue in human subjects and to examine the relationship between baseline concentrations of these carotenoids in plasma and normal cervical tissue in subjects who were being evaluated for possible participation in a diet intervention trial. Subjects were 13 women aged 19–41 yr. With the use of HPLC methodology, plasma concentrations of α-carotene, β-carotene and β-cryptoxanthin were determined with UV/visible light detection for plasma and electrochemical detection for cervical tissue. Relationships between plasma and cervical tissue were evaluated with Pearson correlation analysis. Adjusted for plasma cholesterol concentration, plasma α-carotene and β-carotene were correlated with cervical tissue concentrations ($r = 0.91$, $P < 0.001$; $r = 0.90$, $P < 0.001$; respectively). Adjusted for plasma cholesterol concentration, plasma β-cryptoxanthin tended to be correlated with cervical tissue concentrations ($r = 0.62$, $P = 0.058$). These findings suggest that plasma concentrations of α-carotene and β-carotene are good predictors of cervical tissue concentrations of these compounds in human subjects and describe a first step toward demonstrating a biological link between provitamin A carotenoids and cervical cancer in vivo.


KEY WORDS: cervix • carotenoids • humans • electrochemical detection • cervical dysplasia

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The development of cervical cancer is characterized by a progression of normal cervical epithelium to preinvasive cervical lesions, and ultimately, to in situ or invasive carcinoma (Mitchell et al. 1995). Cervical cancer is an important health problem worldwide. In the U.S., 13,700 new cases of invasive cervical cancer are predicted in 1998 with 4900 deaths attributable to this disease (1.8% of all cancer-related deaths in women) (Landis et al. 1998), but these figures do not accurately reflect the extent of the problem. For every case of invasive cervical cancer, there are ~50 cases of abnormal cervical smears that require monitoring and follow-up (Franco 1997), and millions of ablative procedures are performed each year as an approach to treatment.

Human papilloma virus (HPV) is now believed to be a major cause of cervical cancer (Schiffman and Brinton 1995). Results from numerous epidemiologic studies suggest that nutritional factors, particularly carotenoids, are also among the determinants of risk for cervical cancer (Potischman and Brinton 1996), possibly by influencing the progression through the multistep process of cervical carcinogenesis. Topical retinoic acid has been shown to increase the rate of regression of cervical intraepithelial neoplasia (CIN) II (moderate dysplasia) in clinical trials (Meyskens et al. 1994), and all-trans-retinoic acid at physiologic concentrations (1 nmol/L) inhibits the proliferation of HPV-infected cells in culture (Creek et al. 1994). In cervical dysplasia-derived cell lines, the presence of β-carotene (10 μmol/L) was found to induce apoptosis in cervical dysplastic cells via down-regulation of epidermal growth factor receptor protein (Muto et al. 1995). A protective effect of carotenoids against cervical cancer may be mediated by vitamin A–like activities in the affected cervical epithelial cells. Metabolism of β-carotene to retinal has been shown to occur in other peripheral tissues, such as human adipose tissue, primate lung and kidney, and bovine corpus luteum (Schweigert et al. 1988, Wang et al. 1991), in addition to intestinal mucosal cells and hepatocytes. However, a difficult aspect of investigating a possible mechanism in vivo is that only very limited amounts of this target tissue can be biopsied and measured in observational studies and clinical trials.

The purpose of this study was to demonstrate the presence of provitamin A carotenoids in biopsied cervical tissue from human subjects, for which only microsamples can be obtained for analysis, and to examine the relationship between concentrations of these carotenoids in plasma and normal cervical tissue in the subjects.

MATERIALS AND METHODS.

Subjects. Women in this study were consecutive subjects undergoing baseline evaluation for possible participation in a clinical trial to test the efficacy of a carotenoid-rich diet in the treatment of cervical dysplasia. Inclusion criteria were as follows: >18 y of age, recent abnormal cytology results suggesting the possibility of CIN II and premenopausal status. Exclusion criteria included the following: pregnancy, lactation or postmenopausal status. None of the subjects reported use of β-carotene supplements. Demographic data, including age, were obtained from the subjects during recruitment interviews. Procedures for this study were approved by the Human Subjects Committee of the University of California, San Diego, School of Medicine.
**Procedures.** After recruitment, subjects were scheduled for a clinical examination, at which time a blood sample and biopsies of the cervical lesion for histopathological assessment and of normal cervical tissue for carotenoid analysis were obtained. Blood samples were collected by venipuncture into heparin-treated tubes and were protected from light during processing and handling. Plasma was separated by centrifugation at 2300 × g at 4°C for 10 min. Plasma aliquots were stored at ~70°C in cryogenic tubes until lipid extraction and analysis. The cervical tissue biopsies were obtained from the exocervix with the squamocolumnar junction during a standard gynecologic examination using a cervical biopsy punch forceps (Sklar Mini Tischler Delicate Punch, McKesson General Medical, San Diego, CA). Colposcopy was used to visualize the tissue, and the cervix was swabbed with acetic acid solution (50 g/l) and examined to identify areas of normal tissue and CIN lesions to be biopsied. The lesion biopsy was sent to the pathology laboratory for analysis and grading of the dysplasia. The normal tissue sample was rinsed with normal saline (8.5 g sodium chloride/L), and stored at ~80°C in normal saline until analysis.

**Plasma carotenoid and cholesterol analysis.** Plasma carotenoids were separated and quantified by using the HPLC method of Bieri et al. (1985), as modified by Craft et al. (1988), with further modifications to reduce oxidative loss and improve recovery of compounds during analysis (Craft 1992). HPLC analysis was conducted with a Varian Star 9010, 9050 system with variable wavelength UV/visible light detector (Varian Analytical Instruments, Walnut Creek, CA) with wavelength set at 450 nm. The mobile phase was acetonitrile/water (90:10, v/v). Two solvents, A and B, were used: solvent A, 40% solvent B; hold 5 min. Peak identifications were confirmed by injection of authentic standards. We previously demonstrated successful application of this methodology in analysis of various extracts and biological tissues, including a sample of cervix tissue (Ferruzzi et al. 1998). Concentrations obtained with electrochemical and UV/visible light detection have been previously found to be correlated (MacCrehan and Schonberger 1987), but detection limits for the carotenoids are substantially improved with the new technology.

**Statistical analysis.** Initially, descriptive statistics were calculated on all variables, and distributions were examined for normality. Plasma carotenoid concentrations were adjusted for total plasma cholesterol concentrations (as carotenoids:cholesterol) in statistical analysis. Because the distributions for tissue and plasma carotenoid concentrations were skewed and not normally distributed, appropriate power transformations (e.g., log, square root) were investigated for ability to transform the distributions to more closely approximate normality, so that parametric statistics could be computed. Square-root transformation was found to best improve normality; thus this was done before statistical analysis. Correlations between the plasma and cervical tissue concentrations of α-carotene, β-carotene and β-cryptoxanthin were then examined using Pearson product-moment correlation analysis. All analyses were performed using the SPSS for Windows (version 6.0, SPSS, Chicago, IL). Values are means ± SEM.

**RESULTS**

Subjects recruited were 13 women aged 30 ± 2 y (range 19–41 y). Table 1 lists the medians and ranges of α-carotene, β-carotene and β-cryptoxanthin concentrations in plasma and cervical tissue of the subjects. Amounts of cervical tissue that were available for the carotenoid analysis ranged from 5.6 to 62.4 mg; half of the samples weighed 12 mg, reflecting the inherent variability in the amount of tissue that is comfortably removed during a clinical procedure. Total plasma cholesterol concentrations were 5.04 ± 0.26 mmol/L. Pearson correlation analysis revealed significant correlations between tissue and plasma concentrations of α-carotene, β-carotene and β-cryptoxanthin, as shown in Figure 1. The correlation between β-cryptoxanthin concentrations of cervical tissue and plasma, adjusted for plasma cholesterol concentrations, was marginally significant (r = 0.62, P = 0.058).

**DISCUSSION**

Demonstrating a causative relationship between dietary constituents associated with cancer risk in epidemiologic stud-
ies, such as carotenoids and cervical cancer, and elucidating the underlying mechanism are challenging tasks in clinical studies. However, establishing this biological link is important and necessary (Greenwald 1996, Weinstein 1991). Biological action depends on the presence of the protective compound in the target tissue at the crucial point in the pathophysiologic process, which is the focus of this study. We found the provitamin A carotenoids to be measurable in very small cervical tissue biopsy samples using new detection methodology. Also, we found α-carotene and β-carotene to be present in cervical tissue at concentrations that were highly correlated with the concentrations observed in plasma samples.

Previous studies of carotenoids in cervical tissue have involved quantifying these compounds in surgically excised specimens (Palan et al. 1994) and exfoliated cervicovaginal cells (Manetta et al. 1996, Mikhail et al. 1994, Palan et al. 1992). These approaches permit the use of conventional HPLC detection methodology (i.e., UV/visible light) to quantify carotenoids because relatively large amounts of tissue and cells can be obtained. Clearly, surgical removal of the uterine cervix would not be appropriate in a clinical trial because this does not permit serial analyses to examine effects of intervention. Exfoliated cell collection has limited tissue site specificity, which is necessary in the investigation of etiologic mechanisms in a prospective study. The amount of cells that can be removed directly from the exocervix with cytologic scraping or brushing is limited, which necessitates the collection of vaginal epithelial cells as well; thus the sample is described more accurately as cervicovaginal epithelial cells (Manetta et al. 1996). The small amount of tissue that is typically biopsied for histopathologic examination of cervical tissue has historically limited the ability to quantify carotenoids, until the availability of electrochemical detection methods (Ferruzzi et al. 1998).

Concentrations of the provitamin A carotenoids that were observed in biopsied cervical tissue were within the ranges that have been observed in other human tissues for which the biopsy sample size has allowed analysis using conventional methods. For example, Pappalardo et al. (1997) collected colorectal tissue via colposcopy in 18 subjects with and without colonic polyps or cancer and reported β-carotene concentrations at recruitment into an intervention trial to average 0.03 – 0.07 nmol/g in that tissue across their study subgroups. Oral mucosal samples were obtained by punch biopsy in 28 subjects in a study by Brandt et al. (1994), in which β-carotene concentration was found to average 2.00 nmol/g. Peng et al. (1995) obtained skin biopsies from 96 subjects via punch biopsies and observed β-carotene concentrations in that tissue to average 0.10 – 0.18 nmol/g across their study subgroups.

We also examined correlations between plasma and biopsied peripheral tissue concentrations of the provitamin A carotenoids. In the study by Brandt et al. (1994), serum β-carotene was found to account for only 38% of the variance in mucosal tissue concentrations of biopsied samples, suggesting a substantially weaker relationship between circulating and peripheral pools than was observed in this study. In comparison, good correlations between plasma and skin biopsy β-carotene concentrations (r = 0.75) were found by Peng et al. (1995). A high correlation for α-carotene was also observed in the study by Peng et al. (1995) (r = 0.66), and a somewhat lower correlation (although significant) between plasma and skin β-cryptoxanthin (r = 0.59) concentrations, similar to our observations. The present study group was comprised entirely of premenopausal women; thus greater homogeneity of the population may have contributed to the improved correlations between plasma and peripheral tissue concentrations that were observed.

In conclusion, this study describes a first step toward demonstrating a biological link between provitamin A carotenoids and cervical cancer in vivo. The use of electrochemical detection technology enabled the quantification of carotenoids in biopsied cervical tissue samples, which is the target tissue under study of mechanism and can be collected at specified intervals in a clinical trial. Plasma and cervical tissue concentrations of α-carotene and β-carotene were highly correlated, suggesting that plasma concentrations of these compounds are predictive of amounts in the target tissue.
LITERATURE CITED


