Short Communication

Concentrations of Carotenoids, Tocopherols, and Retinol in Paired Plasma and Cervical Tissue of Patients with Cervical Cancer, Precancer, and Noncancerous Diseases[1]

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Abstract

Paired blood (collected after an overnight fast) and cervical tissue (cancerous, precancerous, and noncancerous) samples were obtained from 87 patients (age, 21–86 years) who had a hysterectomy or biopsy due to cervical cancer, precancer (cervical intraepithelial neoplasia I, II, and III), or noncancerous diseases. The samples were analyzed using high-performance liquid chromatography for 10 micronutrients (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene, *cis*-β-carotene, α-tocopherol, γ-tocopherol, and retinol). The results indicated that: (*a*) among the three patient groups, the mean plasma concentrations of all micronutrients except γ-tocopherol were lowest in the cancer patients; however, the mean tissue concentrations of the two tocopherols and certain carotenoids were highest in the cancerous tissue; and (*b*) among the 10 micronutrients, only the concentrations of β-carotene and *cis*-β-carotene were lower in both the plasma and tissue of cancer and precancer patients than in those of noncancer controls. These results suggest that: (*a*) not all of the micronutrient concentrations in plasma reflect the micronutrient concentrations in cervical tissue; thus, in some cases, it may be necessary to measure the tissue micronutrient concentrations to define the role of the micronutrients in cervical carcinogenesis; and (*b*) maintaining an adequate plasma and tissue concentration of β-carotene may be necessary for the prevention of cervical cancer and precancer.

Introduction

Epidemiological and laboratory studies have indicated that micronutrients, such as carotenoids and vitamins A and E, are potential cancer-preventive agents for certain human cancers including cervical cancer (1–22). These micronutrients may exert their action at the site of target tissues. To define the role of micronutrients in cancer prevention, it is necessary to quantify and compare the micronutrient concentrations in the target tissue with various degrees of the disease, *i.e.*, cancerous, precancerous, and noncancerous tissues.

The objective of the present study was to determine the concentrations of seven carotenoids, two tocopherols, and retinol in the paired plasma and cervical tissue samples obtained from 87 patients with cervical cancer, precancer (CIN[3] I, II, and III), or noncancerous diseases. This study was conducted because: (*a*) many reports have indicated an inverse relationship between the incidence of cervical cancer/precancer and plasma/serum concentrations or intake of carotenoids, vitamin C, vitamin E, and other micronutrients (1–22); and (*b*) we had the opportunity to obtain various cervical tissues from patients with cervical cancer, precancer, or noncancerous diseases. To our knowledge, no cervical tissue concentrations of carotenoids, tocopherols, and retinol had been reported; as such, this study would be significant and should provide important information that was previously unknown.

Materials and Methods

Human Subjects. The subjects recruited were: (*a*) cancer patients who had a hysterectomy due to cervical cancer (n = 27); (*b*) precancer patients who had a cervical biopsy due to mild, moderate, or severe CIN (n = 12, 11, and 10, respectively); and (*c*) noncancer patients who had a hysterectomy because of menorrhagia or other benign conditions (noncancer controls; n = 27). All cases were confirmed pathologically. The subjects were recruited in 1990 and 1991 from the University Medical Center, Tucson Medical Center, and Southern Arizona Surgical Oncology Ltd. (Tucson, AZ) by Drs. J. M. Childers and K. D. Hatch. This study was approved by the Human Subjects Committee of the University of Arizona.

Collection of Blood and Cervical Tissue Samples. Blood samples were collected from each patient after an overnight fast at the time of surgery or biopsy. At each collection, approximately 5 ml of blood were drawn by venipuncture into a foil-wrapped, green-topped tube (heparin) and immediately stored in a refrigerator at 4°C. In addition, three types of cervical tissues, *i.e.*, cancerous tissue from cancer patients, precancerous tissue from precancer patients, and noncancerous tissue from noncancer patients, were also collected. The portion of the tissue for micronutrient analysis was placed in a container at the time of surgery or biopsy and immediately stored in a refrigerator at 4°C. The blood and tissue samples were transported to our analytical laboratory in a cooler within 2 h of collection.

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[3] The abbreviations used are: CIN, cervical intraepithelial neoplasia; HPLC, high-performance liquid chromatography; BHT, butylated hydroxytoluene.

Table 1 Micronutrient concentrations in the plasma of cancer, precancer, and noncancer patients

Micronutrients	Cancer patients (n = 27) (ng/ml)	Precancer patients (n = 33) (ng/ml)	Noncancer patients (n = 27) (ng/ml)
Lutein	54 ± 21 (13–96)[a,b]	99 ± 51 (17–251)[b]	77 ± 50 (26–281)
Zeaxanthin	12 ± 6 (4–26)[b,c]	21 ± 10 (5–47)[b]	17 ± 7 (5–33)[c]
β-Cryptoxanthin	52 ± 21 (18–115)[b,c]	83 ± 41 (9–203)[b]	95 ± 55 (14–231)[c]
Lycopene	172 ± 86 (40–412)[b,c]	331 ± 192 (70–1200)[b]	277 ± 151 (68–755)[c]
α-Carotene	37 ± 30 (9–153)	57 ± 61 (3–307)	55 ± 49 (7–233)
β-Carotene	139 ± 137 (24–707)	172 ± 171 (15–779)	273 ± 408 (18–2101)
cis-β-carotene	10 ± 9 (3–50)	12 ± 12 (2–55)	22 ± 38 (2–200)
α-Tocopherol	7342 ± 2544 (3271–14193)[c]	7692 ± 2284 (3413–13719)	10546 ± 5407 (2388–23674)[c]
γ-Tocopherol	1482 ± 764 (441–3472)	1693 ± 769 (921–5400)	1474 ± 686 (675–3747)
Retinol	288 ± 113 (100–524)[b]	420 ± 118 (205–737)[b]	355 ± 124 (94–560)
Total carotenoids	475 ± 194 (174–1095)[b,c]	775 ± 442 (136–2691)[b]	815 ± 592 (145–3217)[c]
Total micronutrients	9588 ± 2756 (4568–16300)[c]	10580 ± 2902 (4807–18125)	13189 ± 5659 (3693–26326)[c]

[a] Means ± SD (range).
[b] These values have significant difference at $P < 0.05$.
[c] These values have significant difference at $P < 0.05$.

Processing and Storage of Samples. All of the samples were processed under red dim light as described (23, 24). The blood samples were centrifuged at 1200 × *g* for 10 min at 4°C. Plasma was collected, measured in aliquots of 0.25 ml/tube, and stored at −70°C until analysis. The cervical tissues were cleaned (any blood and visible fat were removed), weighed, and then stored at −70°C.

Extraction of Micronutrients from Plasma and Cervical Tissue. Extraction was also carried out under red dim light using our previously published procedure (23, 24).

Plasma. Two hundred and fifty μl of 1% SDS in ethanol containing 0.1% BHT (w/w/v) were added to 0.25 ml of plasma. After mixing, the micronutrients were extracted twice with 500 μl of hexane containing 0.1% BHT (w/v). The dried extract was stored at −20°C until HPLC analysis within 3 days.

Cervical Tissue. BHT (1–2 mg), 400 μl of PBS, and 50 μl of 5% collagenase solution (Type IV; Sigma) were added to approximately 50 mg of cervical tissue in a 2-ml microcentrifuge tube. After mixing, the samples were incubated at 37°C for 1 h and then homogenized on ice using a hand-held polytron tissue homogenizer (model PT 10/35; Brinkmann Instruments, Westbury, NY). After the homogenization, 50 μl of 2% protease solution were added to each tube. The samples were mixed and then incubated at 37°C for 30 min. After the incubation, 500 μl of the SDS-ethanol-BHT solution were added to each tube, and the micronutrients were extracted with hexane as described above. The incubation, homogenization, and extraction were carried out in the same tube to avoid the loss of samples.

HPLC Analysis. HPLC analysis was performed using our previously published procedure (23, 24). Two Novapak C_{18} columns (4 μm; 300 × 3.9 mm; Waters Associates, Milford, MA) connected in series and preceded by a guard column (70 × 2.1 mm; Waters Associates) packed with CO:Pell octadecyl silane (Alltech, Deerfield, IL) were used for the analysis. To separate the 10 micronutrients simultaneously, a gradient mobile phase at a flow rate of 1.3 ml/min was used. Mobile phases A and B consisted of acetonitrile, tetrahydrofuran, methanol, and 1% ammonium acetate in different proportions (23). The total run-time, including reequilibration, was 47 min. The HPLC effluent was monitored simultaneously at 300 (tocopherols), 325 (retinol), and 452 (carotenoids) nm.

With this analytical procedure, the plasma concentrations of β-carotene, α-tocopherol, and retinol analyzed by us in the test serum samples supplied by the National Institute of Standards and Technology (Bethesda, MD) for a quality control/quality assurance study were in agreement with the values reported by the Institute. The coefficient of variation for the analysis of the 10 micronutrients in plasma and cervical tissue over a 1-month period ranged from 3.59–9.09% (23, 24). Also, the recovery of lutein, lycopene, β-carotene, retinol, and α-tocopherol added to the cervical tissue homogenate was 94, 86, 90, 96, and 96%, respectively (24).

Statistical Analysis. Statistical analysis was performed using a SAS statistical package from the SAS Institute (Cary, NC; see Ref. 25). For descriptive purposes, means and SDs were computed. To adjust for skewness in the observed micronutrient concentrations, all analyses were performed using the natural log-transformed values. To assess the difference in the micronutrient concentrations among the diagnostic groups, one-way ANOVA was used. If a significant overall difference was detected between the three groups, Tukey's test was used to determine which pairs of diagnostic groups differed. Because of potential imbalances due to differences in age and smoking status, three-way ANOVA was used after adjustment for potential confounders to assess differences between the diagnostic groups. A level of $P < 0.05$ was set for significance of all tests.

Results and Discussion

The study population consisted of 27 cancer patients, 33 precancer patients, and 27 noncancer patients. The mean ages of the cancer patients and noncancer patients were 49.2 and 48.7 years, respectively; however, the precancer patients were younger (mean age, 31.4 years). The smoking data from the majority of the patients (83% of the patients provided smoking information) indicated that cancer and precancer patients were current smokers in greater proportion (approximately 24 *versus* 12%) than noncancer patients. The precancer patients with CIN I, II, and III were combined into one group, because their plasma and tissue concentrations of the micronutrients were not different.

The micronutrient concentrations in the plasma of the cancer, precancer, and noncancer patients are shown in Table 1. Among the three patient groups, the mean plasma concentrations of all micronutrients except γ-tocopherol were lowest in the cancer patients. The mean plasma concentrations of total carotenoids and total micronutrients (the sum of carotenoids, tocopherols, and retinol) in the three patient groups, in decreasing order, were noncancer > precancer > cancer. The micronutrients that exhibited a significant difference ($P < 0.05$)

Table 2 Micronutrient concentrations in the cancerous, precancerous, and noncancerous tissues

Micronutrients	Cancerous tissues (n = 27) (ng/g wet tissue)	Precancerous tissues (n = 33) (ng/g wet tissue)	Noncancerous tissues (n = 27) (ng/g wet tissue)
Lutein	32 ± 19 (5–81)[a]	22 ± 11 (7–63)	22 ± 10 (4–48)
Zeaxanthin	12 ± 7 (2–31)[b]	7 ± 3 (1–16)[b]	7 ± 5 (2–19)
β-Cryptoxanthin	22 ± 13 (1–45)[b]	15 ± 8 (4–33)[b]	20 ± 13 (4–48)
Lycopene	135 ± 90 (9–314)	134 ± 85 (29–423)	128 ± 86 (34–362)
α-Carotene	24 ± 20 (5–89)	23 ± 16 (6–78)	32 ± 29 (2–134)
β-Carotene	84 ± 64 (6–246)	62 ± 43 (8–226)[c]	124 ± 109 (12–387)[c]
cis-β-carotene	20 ± 16 (1–64)	16 ± 10 (3–55)[c]	37 ± 41 (2–190)[c]
α-Tocopherol	5686 ± 2530 (1629–11653)[b,d]	2898 ± 936 (1263–5472)[b,c]	4165 ± 1511 (1498–7834)[c,d]
γ-Tocopherol	1674 ± 1069 (423–5091)[b,d]	940 ± 328 (324–1556)[b]	1006 ± 525 (344–2264)[d]
Retinol	36 ± 26 (14–132)	41 ± 16 (15–71)	34 ± 14 (4–67)
Total carotenoids	323 ± 181 (32–710)	280 ± 151 (81–883)	374 ± 240 (55–985)
Total micronutrients	7654 ± 3180 (2601–15057)[b,d]	4145 ± 1274 (2162–7242)[b,c]	5579 ± 1532 (2566–8684)[c,d]

[a] Means ± SD (range).
[b] These values have significant differences at $P < 0.05$.
[c] These values have significant differences at $P < 0.05$.
[d] These values have significant differences at $P < 0.05$.

among the three patient groups were lutein (cancer < precancer), zeaxanthin (cancer < noncancer, cancer < precancer), β-cryptoxanthin (cancer < noncancer, cancer < precancer), lycopene (cancer < noncancer, cancer < precancer), α-tocopherol (cancer < noncancer), and retinol (cancer < precancer). The differences remained significant after adjusting for smoking and age.

Table 2 shows the micronutrient concentrations in the cancerous, precancerous, and noncancerous tissue. The mean concentrations of micronutrients other than α-carotene, β-carotene, and *cis*-β-carotene were higher in the cancerous tissue than in the noncancerous tissue and the precancerous tissue. In addition, the mean concentrations of many micronutrients were higher in the cancerous tissue than its adjacent tissue in four of the five cancer patients investigated (data not shown). It seemed that the cancerous tissue was able to accumulate many micronutrients. The accumulation was unexpected and could be due to: (*a*) a higher level of fat in the cancerous tissue; hence, more fat-soluble micronutrients were found in the tissue; (*b*) a rapid growth of the cancerous tissue that required more micronutrients; and/or (*c*) abnormal cellular regulatory mechanism or mechanisms.

Among the micronutrients that showed divergent changes in their plasma and tissue concentrations compared to noncancer controls, α-tocopherol showed the greatest divergence (Tables 1 and 2). Its plasma concentration was significantly lower, but its tissue concentration was significantly higher in the cancer patients than that of noncancer controls. This divergence indicates that not all of the plasma micronutrient concentrations can be used to assess the micronutrient status of cervical tissue, at least in this patient population. Thus, in epidemiological studies and clinical trials, careful interpretations of the plasma micronutrient concentrations in relation to the status of the disease may be warranted.

Among the 10 micronutrients, only the concentrations of β-carotene and *cis*-β-carotene were lower (compared to noncancer controls) in the plasma and in the cancerous and precancerous tissues of both cancer and precancer patients (Tables 1 and 2). This finding is consistent with the previous report that precancer patients had a lower concentration of β-carotene in the cervicovaginal cells than controls (10). These results suggest that maintaining an adequate concentration of β-carotene in the plasma and cervical tissue may be important for the prevention of cervical cancer and precancer.

In summary, we have quantified the concentrations of seven carotenoids, two tocopherols, and retinol in paired plasma and cervical tissue samples of 87 patients. Comparing the plasma and tissue concentrations of cancer, precancer, and noncancer patients, we have found that the concentration of β-carotene was lower in both the plasma and tissue of cancer and precancer patients than in those of noncancer controls. The results suggest that β-carotene may have a role in the prevention of cervical cancer and precancer. In addition, from the comparison, we unexpectedly found that the concentrations of many micronutrients other than α-carotene, β-carotene, and *cis*-β-carotene were higher in the cancerous tissue than in the noncancerous and precancerous tissues. The reason for the higher concentrations and their biological significance is not known and remains to be investigated.

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