ONCOLOGY

Vitamin E and A Contents in Renal Carcinoma Tissue

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UDC 616.61-006.6-008.93:577.161.11]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 290-293, March, 1993
Original article submitted September 9, 1992

Key Words: *vitamins E and A; human renal cell carcinoma; tumor lipids*

An important role of vitamin E in oncogenesis has been demonstrated in a number of experimental studies in laboratory animals. In particular, it was found that chemically induced or implanted tumors of rats, which were given a ration with a normal or high vitamin E content, accumulated the vitamin more actively than the normal homologous tissues [8]. E. A. Neifakh [3] in a study on malignant tumors of mice and rats (ascitic carcinoma, hepatoma, sarcoma) reported 4- to 9-fold higher α-tocopherol concentrations in tumor lipids than in normal tissues. The author also found that the vitamin is actively consumed by the tumors and oxidized into tocopherolquinone. In addition, there was a marked decrease in the tumor lipid peroxidation. Similar data were presented by Burton *et al.* [4] in their study on rat hepatoma. The authors suggested that the accumulation of an antioxidant, α-tocopherol, in malignant tumors might favor their development due to the formation of lipoperoxides, which are known to inhibit cell proliferation. As a result, the division of malignant cells would be relieved of the host control.

With regard to human tumors, the data on their vitamin E content are limited and controversial. The levels of vitamin E in stomach and colon carcinomas have been found to be lower than in the mucosa near the tumor or in the corresponding tissues of healthy persons [7]. The vitamin E level in lymphocytes of patients with chronic lymphocytic leukemia wa reported to be twofold lower than that of healthy controls, whereas in patients with hair cell leukemia it was 1.5-fold higher than in controls [5].

The purpose of our investigation was to estimate the vitamin E content in human renal cell carcinoma (RCC).

MATERIALS AND METHODS

Eighteen kidneys from renal tumor patients, subjected to unilateral nephrectomy, were analyzed. Based on histological data 17 tumors were identified as RCC and characterized by the following cellular patterns: 6 patients had clear cell carcinomas with degree II of malignity, 2 had either polymorphous cell carcinoma or granular cell carcinoma with degree III of malignity, and 9 had mixed carcinomas of which 4 were mainly represented by clear cells and 5 by granular or glandular cells, although clear cells were also present in each specimen. In one case a benign tumor, angiomyolipoma, was diagnosed. Because of the characteristic biochemical features of the clear cell carcinomas (hypernephroma), such as high cellular concentrations of triglycerides, cholesterol esters and glycogen [6], these tumors, as well as mixed tumors with the predominance of clear cells, were treated as

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0007-4888/93/0003-0317$12.50

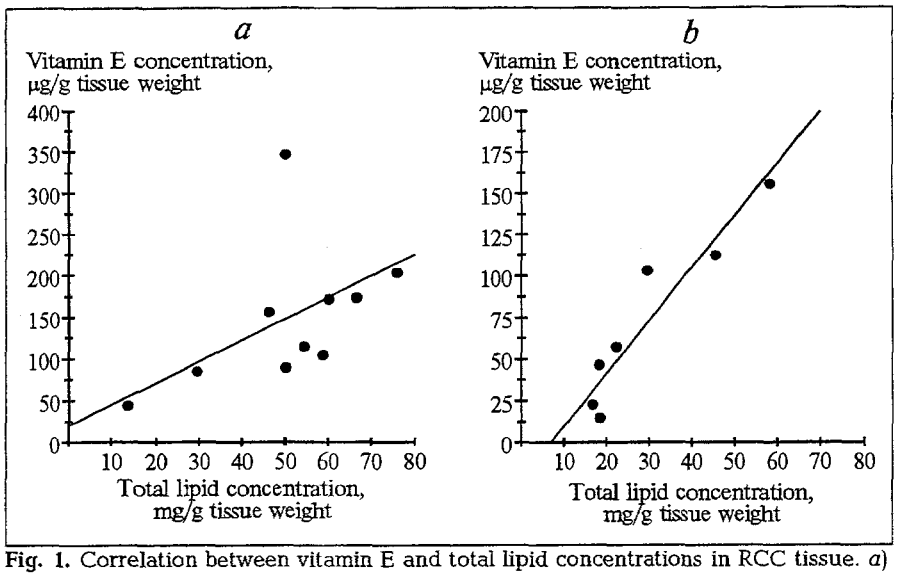

Fig. 1. Correlation between vitamin E and total lipid concentrations in RCC tissue. *a*) tumors of group I; *b*) tumors of group II.

a separate group (group I, 10 cases). Group II included other renal carsinomas (7 cases). Angiomyolipoma was analyzed separately.

Biochemical analyses were carried out using both tumor homogenates and homogenates of "intact" renal tissues, not engaged in the tumorous process. Only those tumor pieces not containing any necrotic sites, cysts, or scars were used. In the case of "intact" renal tissue, the cortex and medulla (the latter without papillae) were investigated separately. Tissue samples were homogenized in 5 volumes of physiological solution (300 mg per 1.5 ml). Vitamin E in 0.75 ml of the homogenate was determined by the spectrofluorometric method [9]. After saponification of lipids, vitamin E was extracted by hexane and the fluorescence was measured in a Hitachi-650-10S spectrofluorometer at 325 nm (emission) and 292 nm (exciting) In 6 experiments (carcinomas as well as "intact" cortex and medulla) vitamin A was determined [10] in the same hexane extracts as were used for vitamin E measurements with the emission and exciting wavelengths 460 nm and 335 nm, respectively. Both the vitamin E and A concentrations were calculated per gram wet weight of tissue and per mg tissue lipids. The total lipid concentrations in the tissue homogenates were determined using the sulfovanillic reagent [1]. Tumor samples prepared for histological assays were fixed in formalin, which had been neutralized and buffered with Lilly byffer, then in Carnoy fluid, and, finally, embedded in paraffin. Tissue slices were stained with hematoxylin-eosin, Sudan III, or toluidine blue.

Student's *t* test, Wilkcoxson's *T* test for paired data, and correlation analysis were used for the statistical processing of the data.

RESULTS

Table 1 shows that the two layers of "intact" kidneys differed from each other in their vitamin E levels: in the medulla vitamin E was significantly higher than in the cortex when calculated both per gram tissue weight and per milligram total lipids. The same differences were observed in the experimental animals. Although RCC primarily derives from the cortical epithelial cells [2], we compared the vitamin E concentrations in tumor tissue and in two layers from a normal kidney. Note the extremely high vitamin E concentrations (calculated per gram tissue) in the tumors of group I, where they were on average 15-fold and 10-fold higher than in the cortex and medulla, respectively.

The total lipid concentrations in the tumor tissue were also markedly increased as compared to the "intact" kidney: 5-fold and 3-fold for groups I and II, respectively. Nevertheless, when calculated per milligram total lipids, the vitamin E concentrations were higher in the group I carcinomas than in either layer of "normal" kidneys and they were higher in

TABLE 1. Content of Vitamin E and Total Lipids in "Intact" Renal, RCC, and Angiomyolipoma Tissue ($M \pm m$)

Parameter	"Intact" tissue (n=17)		p_{2-1}	RCC, group I (n=10)	p_{3-1} p_{3-2}	RCC, group II (n=7)	p_{4-1} p_{4-2}	Angiomyolipoma (n=1)
	cortex	medulla						
Vitamin E, μg/g	9.45±0.49	14.35±0.96	<0.001	152.2±28.9	<0.001 <0.001	71.8±22.2*	<0.01 <0.05	68.4
Total lipids, mg/g	9.67±0.7	7.93±0.47	<0.05	50.6±5.9	<0.01 <0.001	30.1±6.8*	<0.05 <0.01	91.0
Vitamin E, μg/mg lipids	1.07±0.09	1.87±0.15	<0.001	3.14±0.45	<0.001 <0.05	2.17±0.36*	<0.01 n.s.	0.75

Note: * – $p<0.05$ when two RCC groups were compared; n.s. – insignificant difference; in parentheses – number of observations.

TABLE 2. Content of Vitamin A and E in RCC Kidneys ($M \pm m$)

Parameter	"Intact" tissue ($n=6$)		p_{2-1}	RCC ($n=6$)	p_{3-1} p_{3-2}
	cortex	medulla			
Vitamin A, mg/g	0.6±0.12	0.32±0.06	<0.05	0.97±0.23	n.s. <0.05
Vitamin E, mg/g	8.2±0.79	11.2±1.1	<0.05	144.3±52.4	<0.05 <0.05
Total lipids, mg/g	10.9±1.39	7.95±0.97	n.s.	40.6±9.57	<0.05 <0.05
Vitamin A, ng/mg lipids	56.0±5.0	37.0±6.0	<0.01	26.0±5.0	<0.01 n.s.
Vitamin E, mg/mg lipids	0.82±0.14	1.42±0.08	<0.01	3.35±0.77	<0.01 <0.05

the group II tumor than in the renal cortex, indicating an enhanced vitamin E supply of the tumor lipids. It is of interest to compare the above data with the vitamin E concentrations in the rarely encountered benigh renal tumor angiomyolipoma (Table 1). When expressed per gram tissue, the concentrations in the tumor were shown to be higher than in the "intact" kidney; however, because of excessive lipids found in the angiomyolipoma, its vitamin levels, calculated per milligram lipids, did not differ from those of the cortex, while being lower than in any of the carcinomas studied. The mechanism underlying the elevated vitamin E accumulation in renal carcinomas are not yet clear. Correlation analysis of the data obtained revealed a highly significant positive correlation between the vitamin E and lipid concentrations in the group II carcinomas ($r=+0.95$, $p<0.001$) However, the correlations between the above parameters for the group I carcinomas were found to be insignificant ($r=+0.53$, $p>0.05$). It seems that the tumor deposition of the vitamin E proceeds more actively than the accumulation of lipids. Proceeding from the assumption that the vitamin E deposition in malignant cells is probably due to the specific composition of tumor lipids [6], we addressed the question whether another liposoluble vitamin, vitamin A, is also accumulated in renal carcinomas. Both vitamins were tested in cancerous and "intact" tissues from 6 patients (2 of group I and 4 of group II). Since the vitamin A concentrations in the tumor groups I and II did not differ significantly (0.85-1.2 and 0.52-1.9 μg/g tissue, respectively), the data for these two groups were combined for statistical treatment (Table 2).

As in the case of vitamin E, the vitamin A concentrations were different in the renal cortex and medulla, the former containing twice as much vitamin as the latter, i.e., there was a reciprocal vitamin A versus vitamin E distribution. As for the cancerous tissue, its vitamin A concentrations did not differ significantly from those of the renal cortex, while they were 3-fold higher than in the medulla, which is generally characterized by quite a low vitamin A content. This increase is hardly comparable to the much higher elevation of vitamin E in carcinomas, where its concentrations were 18-fold and 13-fold higher than those in the renal cortex and medulla, respectively. When calculated per milligram total lipids, the vitamin A concentrations were lower in carcinomas than in "intact" renal tissue, indicating that no excessive vitamin A deposition in the tumor lipids occurred. In contrast, the vitamin E content of tumor lipids was 2.5-4-fold higher than that of "intact" renal tissue.

Thus, the data obtained provide evidence for a high vitamin E content of RCC regardless of whether the vitamin concentrations are calculated per gram wet tissue or per milligram total tumor lipids. This obsertvation was especially demonstrable when clear cells were predominant. An enhanced vitamin E influx into the lipids of malignant cells seems to have a favorable effect on the growth of RCC, since this vitamin, being a strong antioxidant, would decrease the production of lipoperoxides, thereby suppressing their inhibitory effect on tissue proliferation. We hypothesize that the vitamin E accumulation in RCC may be due to the characteristic features of tumor lipid metabolism rather than to high tumor lipid concentrations. This suggestion is based on the finding that the tumor levels of another liposoluble vitamin, vitamin A, which is not actively involved in lipid metabolism, are not increased when expressed per milligram total tissue lipids.

The above data on the vitamin E content of renal carcinomas are reported for the first time in this investigation. We could not find any related information in the available sources.

The authors are grateful to Dr. A.V.Morozov, Head of the Department of Urology, Hospital 6, Moscow, for providing us with operation material.

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Benz(a)pyrene Induces Squamous-Cell Metaplasia of the Respiratory Epithelium in Explants of Embryonic Mouse Lungs

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UDC 616.24−006.6−02:615.277.4:665.44.015.3−07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, No. 3, pp. 293−295, March, 1993. Original article submitted November 16, 1992.

Key Words: *organ cultures; respiratory epithelium; squamous-cell metaplasia; lung blastomogenesis*

Squamous-cell metaplasia of the respiratory epithelium occurs in the lungs of patients undergoing surgery for malignant and inflammatory diseases of the lungs and in individuals dying from other causes as well [10]. Metaplasia of the respiratory epithelium, in combination with other alterations specific to respiratory epithelium dysplasia, is most frequently found in men with a long history of heavy smoking [6]. Smoking is thought to be related to the preferential development of central lung cancer of a distinct histological type, namely, squamous-cell lung cancer typical for men [3,7,8]. The mortality from lung cancer in Russia and several other countries is increasing in parallel with the spread of the smoking habit among men and women [1,4]. On the other hand, in some countries the level of lung cancer morbidity has dropped following a decrease in the numbers of men and women smokers [8]. A drop in the frequency of squamous-cell dysplasia of the respiratory epithelium in men after quitting smoking has been noted in some reports [6]. The relationship between smoking, frequency of the development of squamous-cell respiratory epithelium metaplasia, and squamous-cell carcinoma of the lungs is consistent with the notion that squamous-cell metaplasia of the respiratory epithelium is one of the stages of development of squamous-cell lung cancer [5]. One of the components of tobacco smoke is benz(a)pyrene (BP), and its carcinogenic properties are thought to be con-

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0007−4888/93/0003−0320$12.50