Short Communication

Decrease of Ornithine Decarboxylase Activity in Premalignant Gastric Mucosa and Regression of Small Intestinal Metaplasia in Patients Supplemented with High Doses of Vitamin E

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Abstract

The effect of high doses of vitamin E (Vit.E; 400 units/day) on ornithine decarboxylase (ODC) activity and regression of small intestinal metaplasia (SIM) was studied in a 1-year double-blind intervention trial. Biochemical and morphological parameters were estimated in 14 evaluable SIM patients of 18 in the Vit.E group and in 16 of 18 intestinal metaplasia patients enrolled in control group (placebo). In the control group, there were no statistically significant changes in Vit.E content in blood plasma, ODC activity, and the rate of SIM in multiple biopsies from antrum gastric mucosa. In the Vit.E group, after 6 and 12 months of intervention, the initial content of Vit.E in blood plasma increased from 6.4 ± 0.9 up to 17.0 ± 1.8 and 21.2 ± 2.3 μg/ml, respectively, and the initial abnormally high activity of ODC, 62.6 ± 7.8 units, decreased by 53 and 65%, respectively. Histological analysis of multiple biopsies, taken from the gastric antrum of patients supplemented with Vit.E, revealed that in 8 of 14 patients (57%) after 6 months and in 10 of 14 patients (71%) after 12 months, no signs of SIM were observed; gastroscopic dye procedure confirmed the regression of SIM in these cases and showed the presence of only small isolated stained areas identified as SIM.

Introduction

Previously, we have found that activity of ODC,[2] a key enzyme regulating the first rate-limiting step of the polyamine biosynthetic pathway, is abnormally high in cases of CAG and intestinal metaplasia, as compared with normal activity (1, 2). Other authors have reported abnormally high ODC activity in patients with premalignant lesions of esophagus (3) and large bowel (4–6).

As has been shown, ODC, if overexpressed, may function as an oncogene (7–11) or may play a significant role in malignant transformation of cells, acting as the transcriptional target of some other oncogenes (10, 12). In *in vitro* systems, the blockage of ODC overproduction by DFMO, an irreversible inhibitor of the enzyme (9), or by some other pathway (11), results in the reversion of the transformed phenotype of cells. In experimental animal models, ODC overexpression plays a critical role in the promotion state of carcinogenesis, and DFMO substantially inhibits tumor development (13, 14). The data suggest that ODC may serve as a suitable intermediate biomarker of premalignant lesions and/or as a target for chemoprevention (15).

Recently, in a randomized placebo-controlled trial, we (16) found that a 1-year supplementation of SIM patients with β-carotene (20 mg/day) resulted in a pronounced decrease of ODC activity, which was accompanied by some regression of SIM (response rate based on 18 evaluable patients was 50%; 95% confidence interval, 26–74%). We (16) proposed that the effect of β-carotene on ODC expression and regression of SIM may be linked intimately with its ability to quench free radicals and scavenge the active oxygen species (17, 18) that take part in the process of tumor promotion (19, 20). Therefore, it was of interest to compare the observed beneficial effect of β-carotene on SIM with any possible effect of Vit.E., which has similar antioxidant properties to β-carotene but differs substantially in its biochemical functions and physiological role (17, 18).

Patients and Methods

Patients. We enrolled 36 CAG patients with SIM, 25 of whom were initially *Helicobacter pylori*-negative and 11 who were successfully treated for *H. pylori* infection; 5 of these 36 patients had SIM with sectorial CM. These patients were selected from among 155 persons with gastrointestinal disorders who visited the Cancer Research Center in Moscow or the Gastrointestinal Unit of the Municipal Hospital in Tula, Russia. All of the patients were fully informed about the aim of the study and agreed to take part in the investigation.

During the primary screening of 155 patients [gastroscopy, determination of *H. pylori* status in one or two biopsies by the 1-min urease test (21), and histological analysis of two biopsies], 51 cases of SIM were revealed. Three SIM patients were excluded from the trial because of the presence of other chronic diseases, treatment with hormonal drugs, and alcohol abuse. Eleven of 23 *H. pylori*-positive SIM patients were effectively treated with a standard chemotherapeutic schedule (de nol, metronidazole, and tetracycline; Ref. 22); clearance of *H. pylori* was confirmed by the 1-min urease test and histological examination performed 3 weeks after treatment.

The design of this intervention study is presented in Fig. 1.

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[2] The abbreviations used are: ODC, ornithine decarboxylase; CAG, chronic atrophic gastritis; DFMO, α-difluoromethylornithine; SIM, small intestinal metaplasia; Vit.E vitamin E; CM, colonic metaplasia.

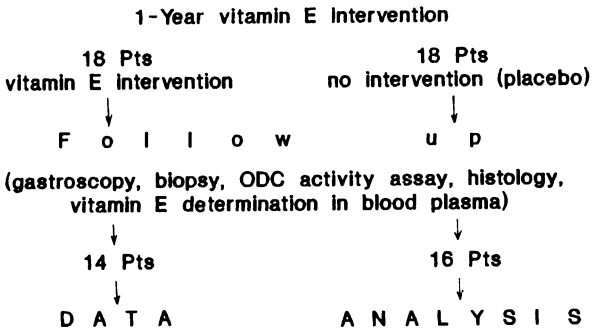

Fig. 1. Intervention study design. Among patients receiving Vit.E, there were 13 patients who were initially *H. pylori*-negative and 5 patients who were efficiently treated for *H. pylori*. In the control group (placebo), there were 12 patients who were initially *H. pylori*-negative and 6 patients who were efficiently treated for *H. pylori*.

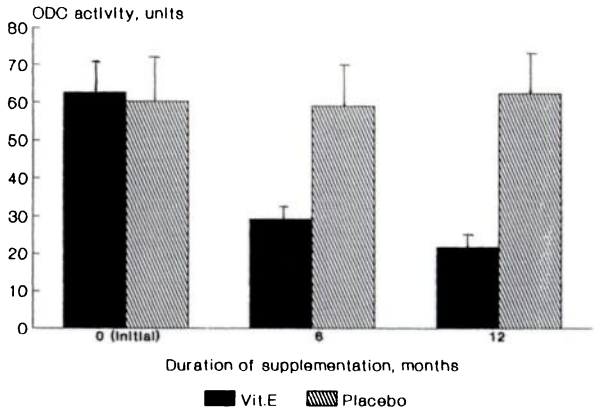

Fig. 2. Effect of Vit.E supplementation on ODC activity in gastric mucosa of SIM patients. *Columns*, means; *bars*, SD.

Thirty-six patients were randomly allocated into two groups. Group 1 (mean age, 54.2 ± 8.6 years; 11 men, aged 39–72 years, and 7 women, aged 40–68 years) received one capsule per day of 400 units of All Natural Vit.E (Vital Life Products; Klaire Laboratories, International, San Marcos, CA), containing about 70% D-α-tocopherol and 30% mixture of D-β-, D-γ-, and D-δ-tocopherols derived from soy oil. Control group 2 (mean age, 56.3 ± 9.7 years; 13 men, aged 45–70 years, and 5 women, aged 36–69 years) received one capsule per day of placebo (refined soy oil). Two patients in the Vit.E group refused to undergo repeated examinations after 6 and 12 months because they felt well, and two patients in the Vit.E. group discontinued supplementation with Vit.E because of intensifying bile reflux, probably as a result of overstimulation of peristalsis by high doses of Vit.E. Only 14 patients in the Vit.E. group and 16 patients in the placebo group were completely followed up during the 1-year trial.

Gastroscopy. Gastroscopy was performed with an endoscopic system Evis-100 (Olympus, Japan). Prior to the start of intervention and at 6 and 12 months during the trial, six or seven biopsies were taken from the antrum at 2–4 cm from the pylorus: two or three for ODC assay and four for histological analysis (one each from the lesser and greater curvatures and two from the lateral walls). At the final gastroscopy, after the biopsies were obtained, the standard endoscopic methylene blue dye procedure, including the removal of gastric mucus with the enzyme pronase in bicarbonate solution (23), was performed for visible detection of SIM and comparison with histological data.

ODC Activity Assay. The standard procedure for assay of ODC activity in human stomach mucosa by sensitive radioisotope methods was described in detail previously (2); 1 unit of ODC activity was taken as a formation of 1 pmol of $^{14}CO_2$/h/mg of protein, determined by the method of Lowry.

Vit.E Determination. Samples of venous blood were taken in the morning after an overnight fast. The concentration of total Vit.E was measured in blood plasma. Samples of plasma (2 ml), stabilized with 0.1 ml of 0.1% β-butoxytoluene in DMSO, were stored in freezer at −20°C for 1 or 2 weeks before the analysis. The levels of total Vit.E in blood plasma were determined by high-performance liquid chromatography (24) using HP 1090M chromatographic system (Hewlett-Packard) equipped with Hypersyl C18 columns and a UV diode array detector.

Histological Analysis. Biopsy specimens taken for histological analysis were fixed in neutralized 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained by standard procedure using H&E. It should be noted that, in many cases, only three of four biopsy specimens taken from each patient were adequate for histological examination. SIM was defined as the presence of Paneth cells as well as nonsecretory columnar cells of metaplastic epithelium, having a prominent strait border of microvilli-like small intestinal epithelium. In a few cases, sectorial CM was observed in patients with SIM. Disappearance of cells diagnostic of SIM or SIM-CM in all evaluable biopsies of individual patient was defined as a regression of premalignant lesion.

Statistical Analysis. Statistical significance of changes in ODC activity after the supplementation of patients with Vit.E was evaluated by two-tailed *t* test. χ^2 method was used for evaluation of statistical significance of SIM regression after the 1-year intervention. Statistical analysis was carried out using Stat-Graphics package.

Results

Initial levels of Vit.E in the blood plasma of patients receiving Vit.E and patients in the control group were 6.4 ± 0.9 and 6.7 ± 1.1 μg/ml, respectively. In the control group, the concentration of Vit.E in blood plasma did not change significantly during the course of placebo supplementation and ranged from 6.5 ± 0.7 to 7.2 ± 1.2 μg/ml. Unlike this, in the Vit.E group after 6- and 12-month supplementation, the blood plasma levels of Vit.E reached 17.0 ± 1.8 and 21.2 ± 2.3 μg/ml, respectively. The effect of high doses of Vit.E on ODC activity in antrum gastric mucosa is presented in Fig. 2. The initial, abnormally high ODC activity (62.6 ± 7.8 units) progressively decreased by 53% after 6 months of Vit.E supplementation and by 65% after 12 months ($P < 0.01$). In the control group, the baseline ODC activity did not change during the course of the 1-year placebo supplementation ($P > 0.05$).

The results of histological examination of multiple biopsies, taken from gastric antrum of SIM patients receiving Vit.E or placebo for 1 year, are summarized in Table 1. Prior to intervention, SIM was observed in most biopsy specimens from all patients in both groups. The prevalence of SIM in total

Table 1 Regression of premalignant lesions and frequency of SIM in multiple biopsies from evaluable SIM patients before and after the 1-year supplementation with Vit.E or placebo

Time of examination	No. of patients with SIM		No. of biopsies from SIM patients			
			Vit.E		Placebo	
	Vit.E	Placebo	Total	With SIM	Total	With SIM
Before treatment	14	16	50	42 (84%)	57	47 (82%)
After 6 months	6[a]	16	48	21 (44%)[b]	59	50 (85%)[c]
After 12 months	4[d]	16	44	8 (18%)[e]	52	45 (87%)[f]

[a] In 8 of 14 patients, no signs of SIM in biopsies were observed; response rate was 57% (95% confidence interval, 31–83%).
[b] $\chi^2 = 15.6$, $P < 0.001$, percentage of change, treatment versus initial level.
[c] $\chi^2 = 0.07$, $P > 0.05$, percentage of change, placebo supplementation versus initial level.
[d] In 10 of 14 patients, no signs of SIM in biopsies were observed; response rate was 71% (95% confidence interval, 47–95%).
[e] $\chi^2 = 38.1$, $P < 0.001$, percentage of change, treatment versus initial level.
[f] $\chi^2 = 0.11$, $P > 0.05$, percentage of change, placebo supplementation versus initial level.

biopsies in the placebo group did not change during 12 months, and in all 16 subjects, SIM (and in 1 patient, SIM-CM) was present at each observation point.

After 6 months of Vit.E supplementation, SIM was detected in only 6 of 14 evaluable patients; in 8 patients (57%), SIM was not observed in any of the available biopsy material. After 12 months of Vit.E supplementation, SIM was detected only in 4 of 14 patients; in 10 patients (71%), no SIM was observed in biopsies. Among these 10 were 2 of 3 SIM patients with sectorial CM, which also disappeared. Ten patients with regression of premalignant lesions included 7 of 13 patients who were initially *H. pylori*- negative and 3 of 5 patients successfully treated for *H. pylori*. The prevalence of SIM in total biopsies of the Vit.E treatment group decreased from 84% to 44 and 18% after 6 and 12 months, respectively ($P < 0.001$).

The *in vivo* staining procedure, conducted at the final gastroscopy, revealed numerous vast patches of stained areas in all patients receiving placebo. Similar observations were made in those patients receiving Vit.E whose biopsies demonstrated SIM histologically. Conversely, in 10 of 14 patients histologically negative for SIM after the 1-year Vit.E supplementation, only small isolated patches of stained area were observed. Comparison of the results of histological analysis of biopsies revealed that, although the regression of SIM produced in some patients taking Vit.E was pronounced, it was not complete.

Discussion

Recently we (16) have shown that the 1-year supplementation of SIM patients with β-carotene (20 mg/day) produced the maximum decrease of abnormally high ODC activity in antrum gastric mucosa of SIM patients by 46%. After 6 and 12 months of Vit.E supplementation, ODC activity decreased by 53 and 65%, respectively. The decrease in ODC activity produced by Vit.E was accompanied also by a partial regression of SIM. Although the number of evaluable SIM patients treated with Vit.E was limited, the decrease in the prevalence of SIM in multiple biopsies after 6 and 12 months of intervention was statistically significant. The partial regression of SIM in many patients of the Vit.E group and the absence of SIM regression in the control group of patients were in accordance with the results of the endoscopic dye procedure, performed at the end of intervention trial.

The exact mechanism of SIM regression produced by Vit.E and β-carotene is not clear. However, it is known that free radicals and active oxygen species can play a significant role in initiation and tumor promotion (19, 20), particularly in process of gastric carcinogenesis (25, 26). Natural antioxidants protect cells from oxidative stress and injurious action of excess free radicals. We hypothesize that SIM regression produced by Vit.E and β-carotene may be intimately linked with the action of antioxidants as antipromoters, blocking the overexpression of ODC, which plays an critical and specific role in tumor promotion. However, it should be noted that, in many respects, the functions and properties of Vit.E and β-carotene essentially differ. For example, β-carotene enhances gap junction communications (18), which inhibit initiated cells from progressing to cells expressing the neoplastic phenotype, whereas Vit.E does not (18). Unlike β-carotene, Vit.E can block the formation of *N*-nitrosocompounds in the lipid phase of gastric mucosa (27). Although Vit.E, like β-carotene, functions in the fat-soluble phase as a singlet oxygen quencher, free radical scavenger, and chain-breaking antioxidant, its kinetic properties and distribution in cells have distinguishing features (17). Vit.E is especially active as an inhibitor of lipid peroxidation in cell membranes, and, unlike β-carotene, is an efficient antioxidant at high oxygen pressure (17). The possible specific mechanisms of Vit.E and β-carotene action in the case of SIM regression require further investigation. In this context, the question of direct or indirect effects of prooxidants on ODC induction and possible participation of other oncogenes in this process is of special interest. Additional larger studies are warranted for confirmation of these findings and for determination of optimal doses and schedules of treatment.

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