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• GASTRIC CANCER •

Effects of vitamin E succinate on the expression of Fas and PCNA proteins in human gastric carcinoma cells and its clinical significance

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Abstract

AIM: To investigate the effects of vitamin E succinate (VES) on the expression of Fas and PCNA proteins as well as its clinical significance in human gastric carcinoma, and to explore the mechanism of VES-induced inhibition of gastric carcinoma cell growth.

METHODS: Immunohistochemical methods were used to detect Fas and PCNA expression both in human gastric cancer SGC-7901 cells treated with VES at different doses and in human gastric carcinoma tissues.

RESULTS: After the SGC-7901 cells were treated with VES at 5, 10, 20 mg/L for 48 h, the positive rates of Fas expression were 16%, 27% and 48%, respectively, significantly increased compared to that of control group (P <0.05); while the positive rates of PCNA expression in groups treated with different doses of VES were 20%, 18% and 7%, respectively, which were significantly decreased compared to that of the control group (P<0.05). In human gastric carcinoma tissues, the Fas positive expression rate was 42.4%(25/59), which declined with the decrease in the degree of tumor differentiation (P<0.05) and with the existence of lymph node metastasis (P<0.001). While the PCNA positive expression rate was 91.5%(54/59), no relationship was observed between PCNA expression and clinicopathologic parameters.

CONCLUSION: VES inhibited the growth of gastric cancer cells by inducing Fas expression and inhibiting PCNA expression. It is, therefore, considered that the expression of Fas and PCNA genes, through tumor cell apoptosis and proliferation, respectively, may be useful as a clinical predictive index in the application of VES to gastric carcinoma therapy, where as Fas may be of more value than PCNA.

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INTRODUCTION

RRR-α-tocopheryl succinate (vitamin E succinate, VES), a derivative of natural vitamin E, has been shown to be a potent growth inhibitor of many kinds of cancer cell types such as human monocytic leukemia cell[1], murine Bl6 melanoma cell[2,3], human prostate cancer cell[4], human breast cancer cell[5,6] and murine EL-4 thymic lymphoma cell[7,8]. Characterization of cellular processes involved in VES antiproliferative effects have shown that VES inhibits tumor cell growth by a variety of mechanisms, including cell cycle blockage[9,10], DNA synthesis arrest[11], induction of differentiation[12-14], and triggering of apoptosis[15-19]. Meanwhile, VES is also noteworthy because of its non-toxic and non-inhibitory effects on normal cell types[20], indicating that VES can be used as a chemopreventive/ chemotherapeutic agent against tumors.

Gastric cancer is one of the most common malignant tumors in China[21-29]. Our previous studies found that VES could inhibit human gastric cancer SGC-7901 cell growth by blocking cell cycle, arresting DNA synthesis and triggering apoptosis[30-32]. In addition, our *in vivo* research demonstrated that VES inhibited benzo (a) pyrene (B (a) P)-induced forestomach carcinogenesis in female mice[33]. In this study, the expression of Fas and PCNA both in SGC-7901 cells treated with VES and in human gastric carcinoma tissues was investigated further for its clinical significance. It may provide reference indices for VES application in clinical therapy.

MATERIALS AND METHODS

Cell culture and treatment

Human gastric cancer cell lines SGC-7901 were maintained in RPMI 1640 medium supplemented with 100 mL/L fetal calf serum (FCS), 100 kU/L penicillin, 100 mg/L streptomycin and 2 mmol/L L-glutamine, incubated in a humidified atmosphere containing 50 mL/L CO_2 at 37 ℃. The SGC-7901 cells were collected after incubation for 48 h in the presence of VES at 5, 10 and 20 mg/L and of ethanol at 1 mL/L as the control group (VES was dissolved in absolute ethanol and diluted in RPMI 1640 complete condition media correspondingly to a final concentration of VES and 1 mL/L ethanol). The cells were then fixed in 40 g/L formaldehyde and embedded in paraffin.

Clinical materials

Fifty-nine paraffin-embedded specimens of gastric cancer were obtained from the pathological laboratory of the First Affiliated Hospital of Harbin Medical University. These specimens were from resections of gastric cancer in this hospital from 1991 to 2001. Of these, 35 were from males, and 24 from females and the age of the patients varied from 31 to 77 years. These specimens had been histologically classified in which 7 were highly to moderately differentiated adenocarcinomas, 52 were poorly differentiated. Thirty-three tumors had invaded the serosa by histology, and 22 cases had local lymph node metastasis.

Reagents and methods

VES was purchased from Sigma Co. Ltd. RPMI 1640 medium was obtained from Gibco BRL. Antibodies against either PCNA or Fas and SP immunohistochemical reagent kit were purchased from Beijing Zhongshan Biotechnology Co. Ltd.

Serial sections of 4 μm thick were sliced from each of the

cell and clinical tissue blocks. These were deparaffinized and rehydrated, and immersed in 30 mL/L H_2O_2 for 10 min to remove the endogenous peroxidase activity. The sections were further incubated with 100 mL/L normal goat serum for 1 h to reduce nonspecific binding. They were, then, incubated with either primary Fas or PCNA antibody at 4 ℃ for 12-16 h. All sections were washed in phosphate buffer solution (PBS) (0.01 mol/L, pH7.2). Afterwards, they were sequentially incubated with biotin conjugated secondary antibody, and avidin biotin enzyme reagent. Staining was done by first immersing the slides in 3, 3' -diaminobenzidine tetrahydrochloride (DAB). All slides were counterstained with haematoxylin/methyl-green, dehydrated and mounted. PBS substituted for the primary antibody was used as the negative control. For the slides initially incubated with primary Fas antibody, if the proportion of positively stained cells was more than 10%, it was considered as a Fas positive sample. For the slides initially incubated with primary PCNA antibody, if the proportion of positively stained cells as more than 50%, it was considered as a PCNA positive sample.

Statistics

The data of Fas and PCNA expression were analyzed by *t*-test. Correlations between Fas and PCNA expression and clinicopathologic parameters were examined using c^2 test. $P<0.05$ was considered to be statistically significant.

RESULTS

The effect of VES on Fas expression in SGC-7901 cells

The Fas positive expression appeared as brown granules located in the membrane and cytoplasm, and the cell nucleus appeared green after counterstaining with methyl green (Figure 1). After the SGC-7901 cells were treated with VES at 5, 10, 20 mg/L and ethanol at 1 mL/L for 48 h, the Fas positive rates were 16%, 27%, 48% and 10%, respectively (Figure 3). The positive rates for the VES treated groups at different doses were significantly increased compared to that of the control group ($P<0.05$), with an evident dose-effect relationship.

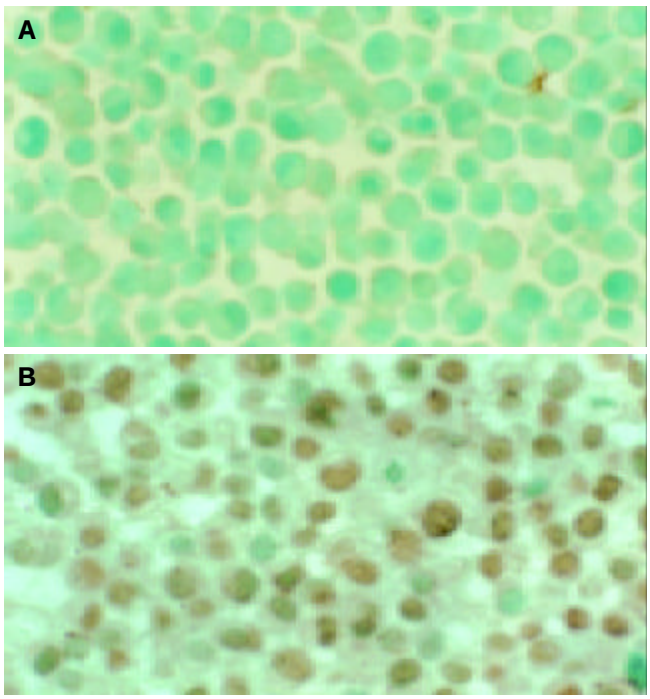

Figure 1 Effects of VES on the expression of Fas protein in SGC-7901 cells. A: control group (×200), B: 20 mg / L VES treated for 48 h (×200).

Effects of VES on PCNA expression in SGC-7901 cells

In contrast to Fas positive expression, the positive PCNA brown stained granules were in the nucleus, while the negative cell nucleus appeared blue after counterstaining with hematoxylin (Figure 2). PCNA positive expression rates, when the cells were treated with VES at 5, 10, 20 mg/L for 48 h were 20%,18% and 7%, respectively (Figure 3), and in the control group 28%. The positive rates of the cells treated with VES at different doses were significantly decreased compared to that of the control group ($P<0.05$), with an evident dose-effect relationship.

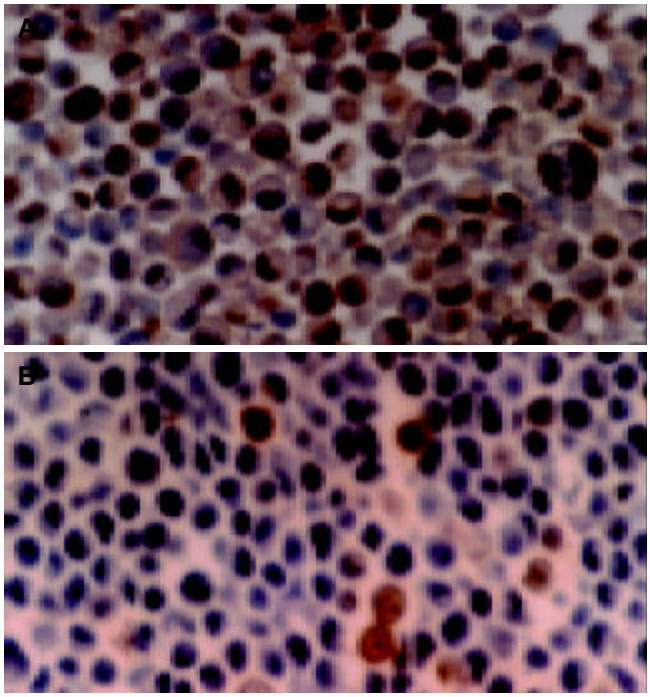

Figure 2 Effects of VES on the expression of PCNA protein in SGC-7901 cells. A: control group (×200), B: 20 mg / L VES treated for 48 h (×200).

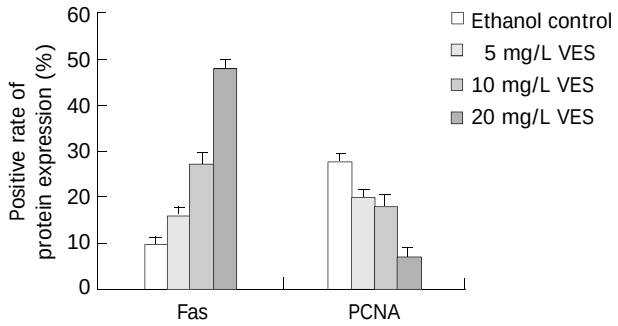

Figure 3 Effects of VES on the expression of Fas and PCNA proteins at different doses.

Expression of Fas in clinical pathological tissues of gastric carcinoma

As shown in Figure 4A, the expression of Fas protein in normal gastric tissues was stronger, for there were many brown stained granules in the membrane and cytoplasm of both glandular and interstitial cells. In the gastric carcinoma tissues, the Fas expression was decreased (Figure 4B) or even negative, the Fas positive rate was only 42.4%(25/59).

Expression of PCNA in clinical pathological tissue of gastric carcinoma

There were brown stained granules located in the nuclei of

PCNA positive cells, while the negative cell nucleus appeared blue after counterstaining with hematoxylin (Figure 5). The positive expression rate of PCNA in normal gastric tissues was not high; the stained cell rate was 49.6%. The stained cell rate in gastric carcinoma tissues was between 33-99%, and the PCNA positive rate was 91.5%(54/59).

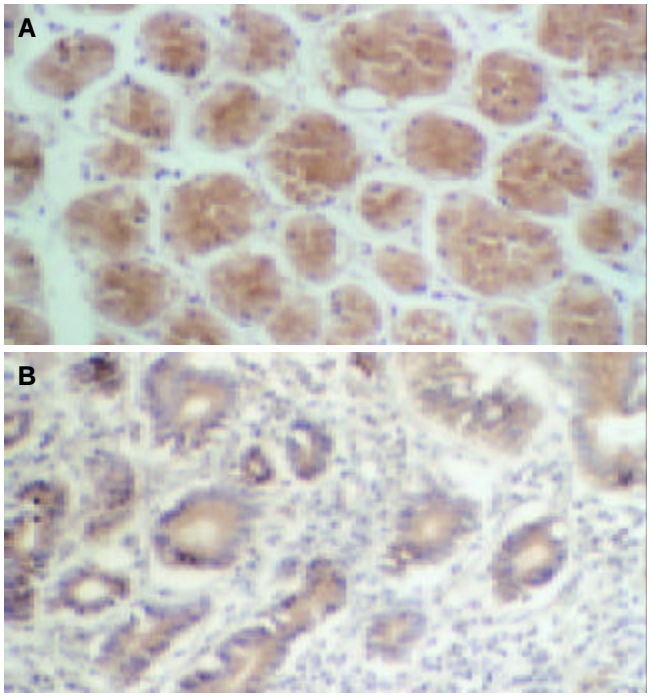

Figure 4 Expression of Fas protein in human normal gastric tissues and gastric carcinoma tissues. A: normal gastric tissue (×200), B: gastric carcinoma tissue (×200).

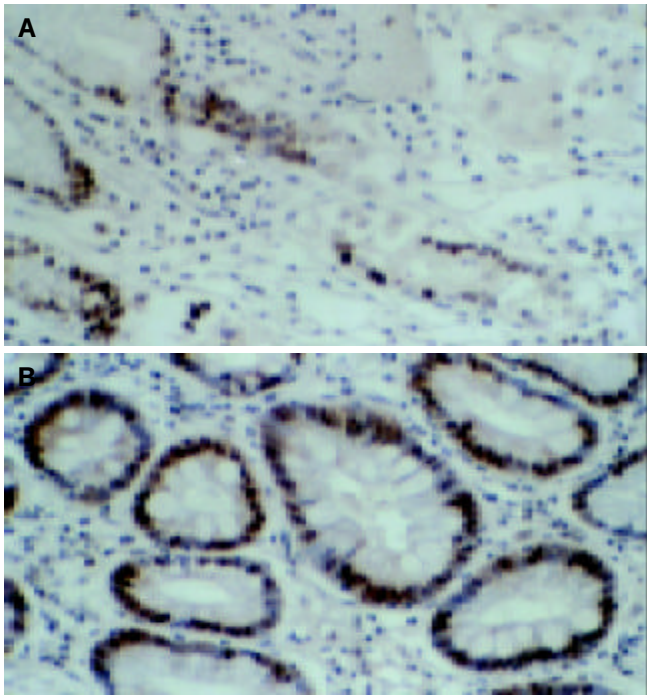

Figure 5 Expression of PCNA protein in human normal gastric tissues and gastric carcinoma tissues. A: normal gastric tissue (×200), B: gastric carcinoma tissue (×200).

Correlation of clinicopathological parameters with expression of Fas and PCNA in gastric carcinoma tissues

As shown in Table 1, the rates of Fas and PCNA protein expression were not correlated with either the patient' s age or sex (P>0.05). The immunoreactivity of Fas was significantly associated with tumor differentiation and lymph node metastasis. Among the 59 cases of gastric carcinoma, Fas positive expression rate in the 7 well-differentiated tissues (85.7%) was obviously higher than that in the 52 cases that were poorly differentiated tissues (36.5%)(P<0.05). The Fas positive expression rate in the group with lymph node metastasis was 13.6%(3/11), lower than in that without lymph node metastasis (P<0.01). PCNA positive expression was not associated with clinicopathologic parameters (P>0.05).

Table 1 Correlation of clinical pathological parameters with expression of Fas and PCNA in clinical gastric carcinoma tissues

Pathologic parameters	*n*	Fas expression		PCNA expression	
		+	(%)	+	(%)
Age (yr)					
⩽45	11	4	(36.3)	11	(100)
>45	48	21	(43.7)	43	(89.5)
Sex					
Male	35	17	(48.5)	30	(85.7)
Female	24	8	(33.3)	24	(100)
Tumor differentiation					
Well-moderate	7	6	(85.7)	5	(71.4)
Poor	52	19	(36.5)[a]	49	(94.2)
Tumor invasion					
Muscularis	26	14	(53.8)	24	(92.3)
Serosa	33	11	(33.3)	30	(90.9)
Node metastasis					
(+)	22	3	(13.6)	22	(100)
(-)	37	22	(59.6)[b]	32	(99.8)

[a]P<0.05, [b]P<0.01.

DISCUSSION

The mechanisms of VES-induced inhibition of tumor cell growth have been a topic of great interest. Turley *et al*[34] observed that the expression of Fas, a cell surface receptor, was increased after treatment of breast cancer cells with VES. They also observed that VES-induced apoptosis in breast cancer cells was inhibited when using Fas neutralizing antibody or transfecting Fas antisense oligonucleotides to cancer cells. Since then, other studies have shown that Fas-mediated apoptosis may be another important pathway by which VES inhibits tumor cell growth[35-37].

Fas/APO-1, a 45 ku type I transmembrane protein, belongs to the nerve growth factor (NGF)/tumor necrosis factor (TNF) receptor superfamily. As a member of the five death domain-containing receptors, Fas initiates a signal-transduction cascade leading to programmed cell death[38-46]. In recent studies, Fas protein expression has been identified in various organs. It was reported that Fas antigen expression could be related to lymph node status and clinical stages in cancer patients. The rate of Fas antigen expression was significantly higher in gastric carcinoma tissues without lymph node metastasis than in those with lymph node metastasis, and also higher in clinical stages I and II than in clinical stages III and IV of gastric carcinoma. Moreover, the prognosis of patients with negative expression of Fas was worse[47,48]. In this study, we determined the Fas expression both in SGC-7901 cells treated with VES and in human gastric carcinoma tissues. The data showed that after 48 h of VES treatment, the expression of Fas protein was evidently increased with a marked dose-dependent relationship compared to that of the control group, indicating that Fas signal

pathway was involved in VES-triggered apoptosis. In addition, the expression of Fas protein in gastric carcinoma tissue was depressed with the decrease of tumor differentiation degree and with the existence of lymph node metastasis. These results are in accordance with the reports mentioned earlier. It suggested that Fas protein expression may be one of indices to predict prognosis of patients with gastric carcinoma, thus establishing a theoretical foundation for using Fas protein expression to assess the effect and prognosis of VES application in clinic therapy.

PCNA functions as a cofactor for DNA polymerase δ. It is associated with DNA repair in both S phase and DNA synthesis phase. As an index of cellular proliferative status, PCNA was determined in various lesions. The overexpression of PCNA with high frequency was usually used as a reliable marker for assessment of tumor progression, premalignant evolution and clinical prognosis of patients with various malignancies[49-59]. Our *in vitro* results showed that VES at different doses could decrease the PCNA expression in SGC-7901 cells to different degrees, indicating that the inhibition of the growth of human gastric carcinoma by VES might be associated with its depression of PCNA protein, decrease in DNA-polymerase δ activity and interference of DNA synthesis. Additionally, the expression of PCNA in clinical gastric carcinoma tissue was higher than that in normal gastric tissues, but we failed to demonstrate the correlation between PCNA expression and clinicopathological parameters in gastric carcinoma in this study, which was conflicted with the reports about PCNA expression in gastric carcinoma[60,61] and the reports that PCNA could be an effective indicator of prognosis for tumor. Based on the *in vitro* results that VES has effects on PCNA expression in gastric carcinoma cells in our study and the reports we have mentioned, we presumed that the conflicting results in our study might be related to fewer cases we have collected, such as only seven cases of highly-moderately differentiated carcinoma.

With the progress of tumor research, it has been realized that the tumorigenesis is a result of imbalance between cell proliferation and apoptosis. On the one hand, cells proliferate massively; on the other hand, cellular apoptosis is inhibited. Fas and PCNA are the two genes closely related to apoptosis and proliferation. Our study indicates that both of them play important roles in the inhibitory effects of VES on gastric carcinoma cells *in vitro*. But for evaluating the effect of VES on gastric carcinoma tissue, Fas, as an effective predicting index for VES application to gastric carcinoma therapy may be of more value than PCNA.

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