P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wcjd@public.bta.net.cn www.wjgnet.com

World J Gastroenterol 2002;8(5):782-786
World Journal of Gastroenterology
 ISSN 1007-9327

• GASTRIC CANCER •

The effects of vitamin E succinate on the expression of *c-jun* gene and protein in human gastric cancer SGC-7901 cells

Yan Zhao, Kun Wu, Wei Xia, Yu-Juan Shan, Li-Jie Wu, Wei-Ping Yu

Yan Zhao, Kun Wu, Wei Xia, Yu-Juan Shan, Li-Jie Wu, Department of Nutrition and Food Hygiene, Public Health School, Harbin Medical University, Harbin 150001, Heilongjiang Province, China
Wei-Ping Yu, Genetics Institute, Texas University of USA, Austin, USA
Supported by National Natural Science Foundation of China, No. 39870662
Correspondence to: Prof. Kun Wu, Department of Nutrition and Food Hygiene, Public Health School, Harbin Medical University, Harbin 150001,Heilongjiang Province,China. wukun@public.hr.hl.cn
Telephone: +86-451-3648665
Received 2002-03-11 **Accepted** 2002-04-20

Abstract

AIM: To investigate the effects of vitamin E succinate (VES) on the expression of *c-jun* gene and protein in human gastric cancer SGC-7901 cells.

METHODS: After SGC-7901 cells were treated with VES at different doses (5,10,20 mg·L^{-1}) at different time, reverse transcription-PCR technique was used to detect the level of *c-jun* mRNA; Western Blot was applied to measure the expression of *c-jun* protein.

RESULTS: After the cells were treated with VES at 20 mg·L^{-1} for 3 h, the expression rapidly reached its maximum that was 3.5 times of UT control (P<0.01). The level of *c-jun* mRNA was also increased following treatment of VES for 6 h. However,the expression after treatment of VES at 5 mg·L^{-1} for 24 h was 1.6 times compared with UT control (P<0.01). Western blot analysis showed that the level of *c-jun* protein was obviously elevated in VES-treated SGC-7901 cells at 20 mg·L^{-1} for 3 h. The expression of *c-jun* protein was gradually increased after treatment of VES at 20 mg·L^{-1} for 3, 6, 12 and 24 h, respectively, with an evident time-effect relationship.

CONCLUSION: The levels of *c-jun* mRNA and protein in VES-treated SGC-7901 cells were increased in a dose- and time-dependent manner; the expression of *c-jun* was prolonged by VES, indicating that *c-jun* is involved in VES-induced apoptosis in SGC-7901 cells.

Zhao Y, Wu K, Xia W, Shan YJ, Wu LJ, Yu WP. The effects of vitamin E succinate on the expression of *c-jun* gene and protein in human gastric cancer SGC-7901 cells. *World J Gastroenterol* 2002; 8(5):782-786

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 various cancer cell types *in vitro* and *in vivo*[1-7]. Growth inhibition by VES is attributed to cell cycle blockage[4,8-10], induced cellular differentiation[11,12], increased expression of biologically active transforming growth factor-βs (TGF-βs) and their type Ⅱ cell surface receptors[1,13,14] and the induction of apoptosis[15-18]. VES is noteworthy not only for its antiproliferative effects on tumor cells, but also for its non-toxic effect on normal cell types.

Gastric cancer is one of the most common tumors in China[19-28]. Up to date, the exact mechanisms of tumorigenesis is still unclear, but our previous studies showed that VES can block cell cycle, arrest DNA synthesis and induce apoptosis in human gastric cancer SGC-7901 cells, therefore inhibiting the cell growth[29-32]. In addition, our *in vivo* research in our laboratory demonstrated that VES inhibited benzo(a)pyrene (B(a)P)-induced forestomach carcinogenesis in female mice[33]. The exact mechanisms of apoptosis are not clearly known, but we found that VES can secrete and activate biologically active TGF-β and then TGF-β increases the kinase activity of *c-jun* N-terminal kinase (JNK) followed by phosphorylation of *c-jun*, and finally activated *c-jun* triggers apoptosis in human gastric cancer SGC-7901 cells[34]. In this study, the expression of *c-jun* mRNA was detected using reverse-transcription polymerase chain reaction (RT-PCR) technique and the level of *c-jun* protein was measured using western blot in order to further investigate the mechanisms of VES-triggered apoptosis.

MATERIALS AND METHODS

Materials

VES was purchased from Sigma Co. Ltd. RPMI 1640 media and TRIzol total RNA isolation kit were obtained from Gibco BRL, TITANIUM™ one-step RT-PCR kit from Clontech. Inc. *c-jun* (H79) rabbit polyclonal antibody was from Santa Cruz Biotechnologies.

Methods

Cell culture Human gastric cancer cell lines SGC-7901 were maintained in RPMI 1640 medium supplemented with 100mL·L^{-1} fetal calf serum (FCS), 100 kU·L^{-1} penicillin, 100 mg·L^{-1} streptomycin and 2 mmol·L^{-1} L-glutamine under 50 mL·L^{-1} CO_2 in a humidified incubator at 37 ℃. SGC-7901 cells were incubated for different time periods in the presence of VES at 5, 10 and 20 mg·L^{-1} (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^{-1} ethanol), succinic acid, vitamin E and ethanol equivalents as vehicle (VEH) control and condition media only as untreated (UT) control.

RT-PCR After SGC-7901 cells were treated with VES for 3, 6 and 24 h, respectively, total cellular RNA was isolated by using TRIzol Reagent according to the manufacturer's instructions. The concentration and purity of total RNA were determined by DU^{R} 640 nucleic acid and protein analyzer (Beckman, USA). One-step RT-PCR was carried out following the manufacturer's instructions. RT-PCR mixture was heated 1h at 50 ℃ for reverse transcription and 5 min at 95 ℃ for pre-denaturation, then into 34 PCR cycles of 30 s at 94 ℃ for

denaturation, 30 s at 60 ℃ for annealing, 30 s at 72 ℃ for extension in PTC-100 programmable thermal controller (MJ Research, USA). The corresponding fragment of *c-jun* gene was amplified with specific primers synthesized[35]. β-actin gene was designed as an internal standard with purpose to remove false negative outcome (Table 1).

Table 1 Nucleotide sequence and size of the expected PCR products for oligonucleotide primers used for RT-PCR

Genes		Sequence	Size (bp)
c-jun	Upstream:	5'-GGA AAC GAC CTT CTA TGA CGA GCC C-3'	315
	Downstream:	5'-GAA CCC CTC CTG CTC ATC TGT CAG G-3'	
β-actin	Upstream:	5'-GTG GGC CGC TCT AGG CAC CAA-3'	540
	Downstream:	5'-CTC TTT GAT GTC ACG CAC GAT TTC-3'	

The amplified products were seperated in 20 g·L^{-1} agorose gel stained with ethidium bromide. After electrophoresis, the gel was observed and photographed under ultraviolet reflector. The density and area of each band were analyzed using ChemiImager™ 4000 Digital System (Alpha Innotech Corporation, USA).

Western blot SGC-7901 cells treated with VES were harvested, washed in PBS and lyzed in lysis buffer containing 150 mmol·L^{-1} NaCl, 1 mL·L^{-1} NP-40, 5 mg·L^{-1} sodium deoxycholate, 1 g·L^{-1} SDS, 50 mmol·L^{-1} Tris (pH 7.4), 1 mmol·L^{-1} DTT, 0.5 mmol·L^{-1} Na_3VO_4, 10 mmol·L^{-1} phenylmethylsulfonyl fluoride (PMSF), 10 mg·L^{-1} trypsin, 10 mg·L^{-1} aprotinin and 5 mg·L^{-1} leupeptin. Following the centrifugation of 12 000×g for 30 min at 4 ℃, the amount of protein in the supernatant was determined using Biorad DC protein assay. Equal amount of protein was separated on 10 % SDS-PAGE and transferred to nitrocellulose filter (Gibco BRL, USA) overnight. Blocked with 50 g·L^{-1} defatty milk, the filter was incubated with *c-jun* (H79) rabbit polyclonal antibody and horseradish peroxidase-conjugated IgG, finally developed with DAB.

Statistical analysis

The data were expressed as $\bar{x}\pm s$. Statistical analysis was performed using student's *t*-test. $P<0.05$ was considered significant.

RESULTS

Effect of VES on the expression of c-jun mRNA in SGC-7901 cells

1 μg of total cellular RNA from groups of control, succinate, vitamin E, VES at 5, 10 and 20 mg·L^{-1} was added to amplify *c-jun* and β-actin genes by RT-PCR. Baseline expression of *c-jun* mRNA was observed in SGC-7901 cells (Figure 1). After the cells were treated with VES at 20 mg·L^{-1} for 3 h, the expression rapidly reached its maximum that was 3.5 times of UT control ($P<0.01$). The level of *c-jun* mRNA was also increased following treatment of VES for 6 h. However, the expression after treatment of VES at 5 mg·L^{-1} for 24 h was 1.6-fold increase compared with UT control ($P<0.01$), while there was no significant difference between 10 and 20 mg·L^{-1} VES groups and UT control group (Table 2).

Effect of VES on the expression of c-Jun protein in SGC-7901 cells

Western blot analysis showed that the level of c-Jun protein was obviously elevated in VES-treated SGC-7901 cells at 20 mg·L^{-1} for 3 h in a significant dose-dependent manner (Figure 2A, 2B). Meanwhile, compared with the cells in UT control group, the VES-treated cells at 20 mg·L^{-1} exhibited 1.8-, 2.0-, 2.3- and 2.8-fold increases in the expression of *c-jun* protein for 3, 6, 12 and 24h, respectively, with an evident time-effect relationship (Figure 3A,3B).

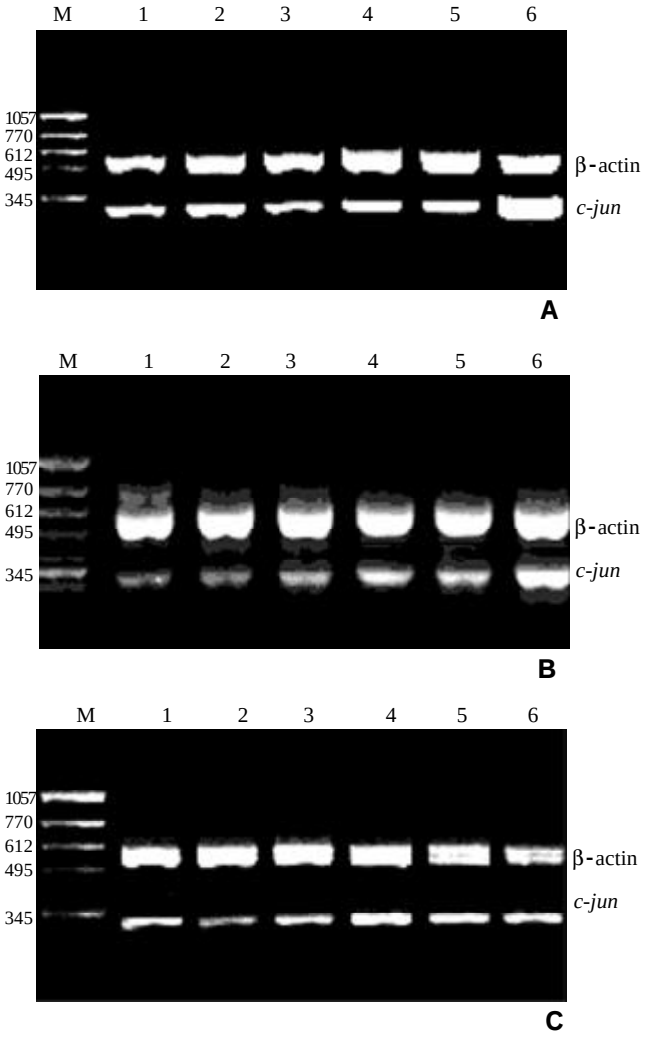

Figure 1 Effect of VES on the expression of *c-jun* mRNA in SGC-7901 cells for different time points by RT-PCR. A: treatment of VES for 3 h; B: treatment of VES for 6 h; C: treatment of VES for 24 h. 1: UT control; 2: succinate; 3: vitamin E; 4: VES at 5 mg·L^{-1}; 5: VES at 10 mg·L^{-1}; 6: VES at 20 mg·L^{-1}; M: molecular weight marker.

Table 2 The relative expression of *c-jun* mRNA in SGC-7901 cells (n=6,$\bar{x}\pm s$)

Groups	Ratio of *c-jun*/β-actin		
	3h	6h	24h
UT control	0.469±0.092	0.432±0.095	0.368±0.104
succinate	0.426±0.082	0.408±0.078	0.361±0.083
vitamin E	0.514±0.101	0.430±0.081	0.367±0.075
5mg·L^{-1} VES	0.550±0.115	0.621±0.086[b]	0.584±0.097[b]
10mg·L^{-1} VES	0.471±0.086	0.584±0.101[a]	0.421±0.077
20mg·L^{-1} VES	1.663±0.109[b]	0.905±0.099[b]	0.411±0.094

[a]$P<0.05$, [b]$P<0.01$, *vs* UT control.

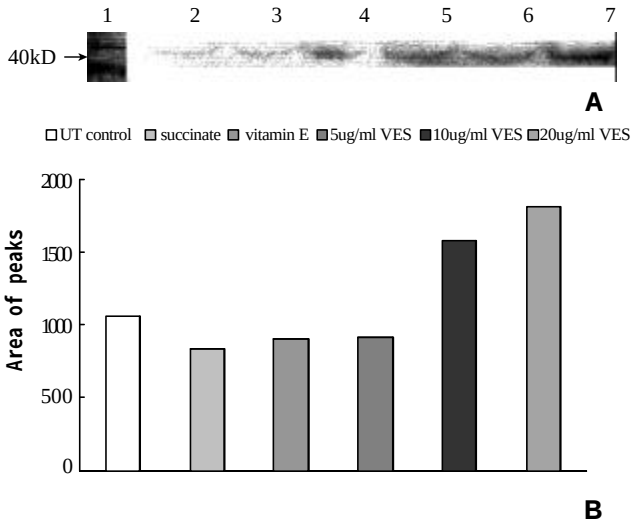

Figure 2 The expression of *c-jun* protein in SGC-7901 cells following treatment of VES for 3h. Lane1: Molecular weight marker; Lane2: UT control; Lane3: succinate; Lane4: vitamin E; Lane5: VES at 5 mg·L^{-1}; Lane6: VES at 10 mg·L^{-1}; Lane7: VES at 20 mg·L^{-1}.

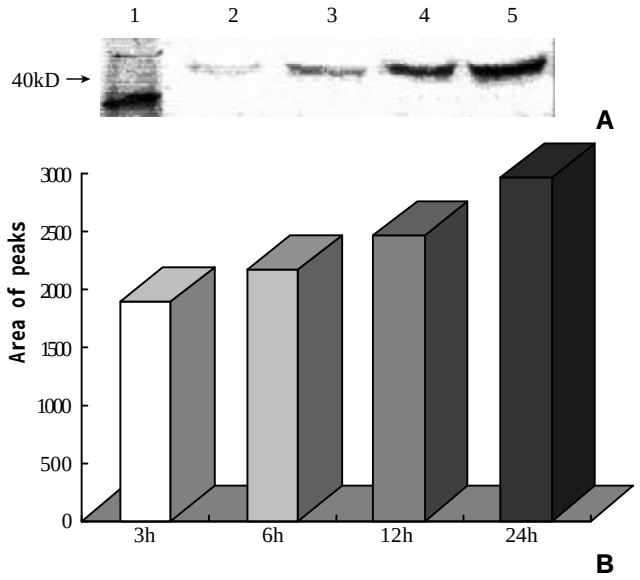

Figure 3 The expression of *c-jun* protein in SGC-7901 cells following treatment of VES at 20 mg·L^{-1} for different time poits. Lane1: Molecular weight marker; Lane2: 3 h; Lane3: 6 h; Lane4: 12 h; Lane5: 24 h

DISCUSSION

The oncogene, *c-jun*, belongs to an immediate early gene and can be rapidly and transiently induced in response to multiple extracellular stimuli[36-38]. The product of *c-jun* gene is a nuclear transcription factor, an important composition of activation protein 1 (AP-1) dimmers, involved in signal transduction and regulation of many kinds of genes[39-41].

Transcription of *c-jun* mRNA rises after exposure of cells to a number of treatment including ultraviolate, irradition, heat shock,H_2O_2,TNF-α and other apoptosis-associated factors[42-46]. In addition to this transcriptional mode of regulation, *c-jun* activity can also be modulated directly at the protein level. In certain cell types, induction of *c-jun* is observed during apoptosis. There is some evidence that prolonged expression of *c-jun* in selected vulnerable cells suggests neuronal cell death[47].

Apoptosis is an innate program of cell suicide that is required for removal of unnecessary or damaged cells from bodily structures. Apoptosis is complex and regulated by a variety of factors[48-58]. Previous studies showed that the induction of apoptosis in tumor cells is one of the important mechanisms of VES-induced cell growth inhibition[59-61]. In the present study, the expression of *c-jun* mRNA and protein was measured in human gastric cancer SGC-7901 cells treated with VES at different doses for different time points. We found that the expression of *c-jun* mRNA was evidently promoted after 3 h of VES treatment at 20 mg·L^{-1} and reduced to the normal level after 24 h of treatment; whereas in the case of VES treatment at 5 mg·L^{-1}, that was also increased after 3 h and remained a high level after 24 h. The data above showed that the *c-jun* activation was enhanced and prolonged by VES, therefore indicating that *c-jun* is involved in VES-triggered apoptosis in SGC-7901 cells. The results from western blot ananlysis showed that the level of *c-jun* protein was elevated following SGC-7901 cells were treated with VES at different doses for 3 h and with VES at 20 mg·L^{-1} for different time in a dose- and time-dependent manner.

The diversity of signals and signaling pathways that are directed toward *c-jun* is also reflected in the biological responses, in which the transcription factors have been implicated. It is reported that the mainly biological functions of *c-jun* are blockage of cell cycle and induction of apoptosis[62-64]. The study presented here demonstrated that VES can obviously increase the expression of *c-jun* mRNA and protein in human gastric cancer SGC-7901 cells, implicating that *c-jun* is involved in VES-induced apoptosis.

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Edited by Pang LH