Inhibitory effects of RRR-α-tocopheryl succinate (VES) on benzo(a)pyrene (B(a)P)-induced forestomach carcinogenesis in female mice

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Abstract

AIM To study the inhibitory effects of VES (RRR-α-tocopheryl Succinate, VES), a derivative of natural Vitamin E, on benzo(a)pyrene(B(a)P)-induced forestomach tumor in female mice.

METHODS The model of B(a)P-induced forestomach tumor was established according to the methods of Wattenberg with slight modifications. One hundred and eighty female mice (6 weeks old) were divided into six groups equally; negative control (Succinic acid), vehicle control (Succinate+B(a)P), high VES(2.5 g/kg.b.w + B(a)P), low VES(1.25 g/kg.b.w + B(a)P) ig as well as VES by ip (20 mg/kg.b.w + B(a)P). Except the negative control group, the mice were administrated with B(a)P ig and corresponding treatments for 4 weeks to study the anti-carcinogenic effect of VES during the initiation period. The experiment lasted 29 weeks, in which the inhibitory effects of VES both on tumor incidence and tumor size were tested.

RESULTS The models of B(a)P-induced forestomach tumor in female mice were established successfully. Some were cauliflower-like, others looked like papilla, even a few were formed into the ulcer cavities. VES at 1.25 g/kg.b.w, 2.5 g/kg.b.w. by ig and 20 mg/kg.b.w. via ip could decrease the number of tumors per mouse (1.7±0.41, 1.6.0±0.34 and 1.1.±0.43), being lower than that of B(a)P group (5.4.±0.32, P<0.05). The tumor incidence was inhibited by 18.2%, 23.1% and 50.0%. VES at 1.25 g/kg.b.w., 2.5 g/kg.b.w. by ig and 20 mg/kg.b.w. via ip reduced the total volume of tumors per mouse (54.8 ± 8.84, 28.4 ± 8.32 and 23.9 ± 16.05), being significantly lower than that of B(a)P group (150.2 ± 20.93, P<0.01). The inhibitory rates were 63.5%, 81.1% and 84.1%, respectively.

CONCLUSION VES has inhibitory effects on B(a)P-induced forestomach carcinogenesis in female mice, especially by ip and it may be a potential anti-cancer agent in vivo.

INTRODUCTION

RRR-α-Tocopheryl Succinate (referred to Vitamin E Succinate, VES) is a derivative of natural vitamin E (RRR-α-tocopheryl)[1]. The interest in this derivative of vitamin E is the fact that it can inhibit the proliferation of a variety of tumor cells in vitro[21]. Tumor cells responsive to VES antiproliferative effects include human monoblastic leukemia cells[3], murine B-16 melanoma cells[4], human prostatic adenocarcinoma cells[5], avian lymphoid cells[6,7], human promyelocytic cells[8], human breast cancer cells[9,10] and murine EL4 T lymphoma cells[11,12].

Although the mechanisms whereby VES inhibits the proliferation of rapidly dividing cells are not well understood, induction of cell cycle blockage[13], increasing secretion and activation of potent negative growth factors, i.e., transforming growth factor-βs and their type II- cell surface receptors[2,7,11,14], and induction of apoptosis[15-17] have been observed in VES-treated cells. Earlier studies in our laboratory using the SGC-7901 cell lines (human gastric cancer cells) as model indicated that VES has antagonistic effects on cell growth and DNA synthesis[18], and can obviously induce apoptosis of SGC-7901 cells[19,20].

To date, VES has not been extensively studied on its antitumor properties as well as its mechanism of action in vivo. Schwartz J and co-workers[21] found that injection of VES directly into the tumor-bearing buccal pouch of hamsters (twice a week for...
four weeks at a dose of 250 µg/injection) caused chemically induced oral epidermoid carcinomas to regress. As VES is found to be a potential chemopreventive and chemotherapeutic agent, it is of interest to ascertain the manner in which tumor growth is inhibited in VES-treated mice and to better understand the relationship between the structure and the function involved. This study characterizes the ability of VES to inhibit B(a)P-induced forestomach carcinogenesis in mice, addresses the involvement of different administration ways in this experiment, and shows that VES is capable of lowering both the tumor incidence and the total tumor size. The inhibitory effects of VES may be attributed to its intact compound.

MATERIALS AND METHODS

Materials

Reagents VES (purity>98%) was obtained from Sigma Co. Ltd, 10% buffered formalin phosphate (10% formalin in neutral phosphate buffer) was purchased from Shanghai Chemical Co. (China), and B(a)P (purity>98%) from Fluka Chemical Co. Ltd (Switzerland).

Animals Female Kunming mice (4-5 weeks old) were purchased from the Animal Center of the Cancer Institute in Heilongjiang Province, China. Fifteen mice were placed in a plastic cage and the animals were maintained under the following standard conditions: 22°C ± 2°C, 45% ± 10% relative humidity, and 12 h light/12 h dark cycles each day. All animals were fed with basic diet (purchased from the Center of Animal Experiment, Heilongjiang, China) and water.

Methods

B(a)P-induced forestomach tumorgenesis Female Kunming mice (6 weeks old) were divided into six groups, each group was composed of 30 mice. Succinic acid, B(a)P and VES were dissolved in corn oil. In group 1 (negative control) and group 2 (vehicle control), the mice were intubated with 1 g/(kg b.w.) succinic acid 4 times per week for 4 weeks. In all groups except group 1, the mice were intubated with 1 mg/mouse B(a)P twice a week for 4 weeks. The mice in group 3 (positive control) were given nothing except B(a)P. Groups 4 and 5 were given 2.5 g/(kg b.w.) or 1.25g/(kg b.w.) VES by the same way 4 times/week for 4 weeks. Group 6 was given 20 mg/(kg b.w.) VES via ip twice a week for 4 weeks. The mice were then sacrificed at week 11, 16 and 29 after the first administration of B(a)P. Buffered formalin-phosphate (10%) was immediately injected into the stomach by intubation into the mouth so that the stomach was distended and fixed. Each stomach was removed and placed on a plastic sheet and the number of tumors in each forestomach was counted. The samples were stored in 10% buffered formalin-phosphate for histological examination.

Tumor volume All tumors were examined with the aid of a magnifying lens, and tumor size was measured. As described previously, tumor volume was determined by measuring the three dimension size of all tumors using the average of the three measurements to calculate radium. Tumor volume was calculated with the formula: volume = 4/3πR³

Histological examination of tumors Tumors found by visual examinations were further confirmed histologically. The stomach samples were excised, fixed in 10% buffered formalin-phosphate, embedded in paraffin and processed for histologic slides with hematoxylin and eosin (HE) staining. Slides were read blindly by a pathologist and the tumors were classified according to the pathological principle.

Statistical analysis The significance of data was determined by t test.

RESULTS

Establishment of B(a)P-induced forestomach tumor model On the basis of previous method with some slight modifications, the model of B(a)P-induced forestomach tumor was successfully established. Treatment of Kunming mice with 1 mg/mouse of B(a)P by ig twice a week for 4 weeks mainly resulted in four forms of tumors (Figure 1). Some bigger ones appeared cauliflower-like, the moderate tumors looked like papilloma, and the smaller ones were usually grain-like. A few tumors had broken into ulcer-like cavities.

The dynamic processes of tumorigenesis in pathology at the week 11, 16 and finally 29 were observed. Before the treatment with B(a)P, the thickness of normal gastric mucosa was uniform with all characterizstics of gastric mucosa in previous studies. The pathological changes at week 11 after the first administration of B(a)P were mainly the hyperplasia of the gastric mucosa, just as what we said “precancerous lesions”, and only a few papillomas could be seen. Under this condition, the thickness of epidermins was not uniform, some positions were very thin, while others were very thick. The nuclei of hyperplastic neoplastic cells were enlarged, round or ovoid with prominent nucleoli. At week 16, the dominant features were papillomas, being more serious than those at week 11, and the proportion of squamous cell carcinomas in the positive group (B(a)P) was 20%, which was more malignant than before. Tumor cells proliferated lumpy into the connective tissues and were polyhedral, with abundant cytoplasm and large nuclei, in which nucleolus was prominent and some were polyhedral. The desmosomes were clear, the mitotic figures and a few keratinized cells could be seen. The typical pathological alterations...
of B(a)P-induced forestomach tumor at the week 29 were that the number of papilloma and squamous cell carcinoma were both increased. The pathological changes of B(a)P-induced forestomach tumor are illustrated in Figure 2 and the dynamic processes of tumorigenesis in each period are shown in Table 1.

Inhibitory effects of VES on B(a)P-induced forestomach tumorigenesis

The treatment with 1mg/mouse- B(a)P twice a week for 4 weeks by ig in group 3 resulted in 100% incidence of forestomach tumors. There was an average of 5.4 tumors/mouse at week 29 of the experiment (Table 2, group 3). Similar results were obtained from group 2. The results in both groups showed that the succinic acid alone had no anticarcinogenic effect. Administration of 1.25 g/(kg b.w.) and 2.5 g/(kg b.w.) VES by ig 4 times/week for 4 weeks inhibited the number of B(a)P-induced forestomach tumors per mouse by 68.5% and 70.4%, respectively (Table 2, groups 4 and 5). When 20 mg/(kg b.w.) VES was injected intraperitoneally into the mice twice a week for 4 weeks, the number of B(a)P-induced forestomach tumors was inhibited by 79.6% (Table 2, group 6). These results suggested that VES could significantly decrease the number of B(a)P-induced tumors per mouse with a dose-dependent manner.

Inhibitory effects of VES on the size of B(a)P-induced forestomach tumor

Administration of 1.25 g/(kg b.w.) and 2.5 g/(kg b.w.) VES by ig significantly decreased the total tumor volume per mouse by 63.5% and 81.1%, respectively. Notably, the treatment with VES via ip at a much lower dose of 20 mg/(kg b.w.) and shorter time of twice a week for 4 weeks showed even higher rate of decreasing the total tumor volume per mouse (84.1%) (Table 3).

Figure 1  The model of B(a)P-induced forestomach tumor.
A. Normal mucosa of forestomach; B. Cauliflower-like tumor; C. Papilla-like tumor; D. Ulcer cavities-like tumor.

Figure 2  Pathological observation of B(a)P-induced forestomach tumor (HE staining) in mice.
A. The normal gastric mucosa ×200; B. The epithelium of gastric mucosa formed papillary projection ×100; C. Squamous tumor cells proliferated into connective tissues and they were polyhedral ×100; D. The mitotic figures (white arrow) in the squamous cell carcinoma ×400
DISCUSSION

The results of our study demonstrated that administration of VES to mice, by both ig. and ip., inhibited B(a)P-induced forestomach tumorigenesis during the initiation period. VES may be useful in the future as a chemopreventive agent of carcinogenesis. A study in vitro indicated that VES is able to inhibit the proliferation of tumor cells without adverse effects on non-tumor cells[27]. Kline et al[2] showed that VES did not inhibit the proliferation of normal murine bone marrow cells and the non-mitogen-stimulated normal avian B and T cells. They also reported that VES can retard the growth of MCF-7, a kind of human breast cancer cell, while it has no inhibitory effects on MCF-10A, a non-tumorigenic mammary cell. Since 1997, we have studied the antagonistic effects of VES on gastric cancer, and found the inhibitory effects of VES on the growth of human gastric cancer cell (SGC-7901), DNA synthesis arrest and induction of apoptosis[18,19]. These findings also supported the possibilities of VES as prospective chemopreventive and chemotherapeutic agent of tumors in the future. VES given intraperitoneally was more effective than given by ig. in lowering both the number and size of B(a)P-induced forestomach tumors even at a much lower dose. Therefore, it seems that VES administrated by ig. may be decomposed by some non-specific esterases which normally exist in stomach, thus losing its original intact structure. VES contains a succinic acid moiety attached to the chroman head structure of RRR-α-tocopherol via an ester linkage. Esterification eliminates the hydroxy moiety that mediates RRR-α-tocopherol’s classical antioxidant properties that prevents VES from acting as an antioxidant, unless the esterified succinic acid moiety is removed by cellular esterases, thereby generating free RRR-α-tocopherol. After VES was proteolyzed into succinic acid and Vitamin E, succinic acid had no inhibitory effects on B(a)P-induced forestomach tumors, as shown in group 2. Previous studies have proved that the inhibitory effect of Vitamin E on

| Table 1 | Pathological alterations in each group at different periods of the experiment |
|---------|-------------------------------------------------------------------------|
| Groups  | Week 11 | Week 16 | Week 29 |
|         | H(%) | P(%) | S(%) | H(%) | P(%) | S(%) | H(%) | P(%) | S(%) |
| 1. Succinic acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. Succinic acid+B(a)P | 100 | 17.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3. B(a)P | 100 | 9.6 | 0 | 100 | 100 | 19.9 | 100 | 100 | 70.4 |
| 4. 1.25g/kgVES+B(a)P | 80.3 | 0 | 0 | 100 | 80.1 | 0 | 100 | 91.8 | 56.9 |
| 5. 2.5g/kgVES+B(a)P | 79.7 | 0 | 0 | 100 | 60.4 | 0 | 100 | 90.3 | 49.8 |
| 6. 20mg/kgVES(ip)+B(a)P | 69.7 | 0 | 0 | 100 | 49.7 | 0 | 100 | 80.7 | 18.4 |

H: Hyperplasia, P: Papillomas, S: Squamous cell carcinoma

| Table 2 | Inhibitory effects of VES on B(a)P-induced forestomach tumorgenesis (x±s) |
|---------|-------------------------------------------------------------------------|
| Groups  | No.of mice | Body weight (g) | Tumors/mouse | % of mice with tumors |
|         | | | | |
| 1. Succinic acid | 12 | 39.5±1.58 | 0 | 0 |
| 2. Succinic acid+B(a)P | 9 | 40.2±1.12 | 5.3±0.48 | 100 |
| 3. B(a)P | 10 | 40.6±1.14 | 5.4±0.32 | 100 |
| 4. 1.25g/kgVES+B(a)P | 11 | 40.1±1.22 | 1.7±0.41(68.5) | 81.8 (18.2) |
| 5. 2.5g/kgVES+B(a)P | 13 | 39.9±1.66 | 1.6±0.34* | 76.9 (23.1) |
| 6. 20mg/kgVES(ip)+B(a)P | 10 | 38.7±1.06 | 1.1±0.43* (79.6) | 50.0 (50.0) |

*Compared with B(a)P group (group 3), P<0.05 (using Student t test)
Numbers in parentheses are % of inhibition compared to group 3.

| Table 3 | Inhibitory effects of VES on the size of B(a)P-induced forestomach tumors (x±s) |
|---------|-------------------------------------------------------------------------|
| Groups  | No.of mice | Vol/tumor(mm³) | Total tumor Vol/mouse(mm³) |
|         | | | |
| 1. Succinic acid | 12 | 0 | 0 |
| 2. Succinic acid+B(a)P | 9 | 31.5±4.51 | 169.5±33.42 |
| 3. B(a)P | 10 | 27.8±8.52 | 150.2±20.93 |
| 4. 1.25g/kgVES+B(a)P | 11 | 31.7±8.62 (0) | 54.8±8.84* (63.5) |
| 5. 2.5g/kgVES+B(a)P | 13 | 17.6±5.45 (36.7) | 28.4±8.32* |
| 6. 20mg/kgVES(ip)+B(a)P | 10 | 21.7±2.13 (21.9) | 23.9±16.05* (84.1) |

*Compared with the B(a)P group (group 3), P<0.01 (using Student’s t test)
ªNumbers in parentheses are % of inhibition of tumors compared to group 3.
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