The Inhibitory Effect of Vitamin E on Arachidonic Acid Metabolism during the Process of Urethane-Induced Lung Tumorigenesis in Mice

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Summary It is known that change in the arachidonic acid metabolism plays an important role in the development of tumors. This study was undertaken to understand the relationship of changes in lipoxygenase, cyclooxygenase and ornithine decarboxylase (ODC) to the inhibitory effect of vitamin E on urethane-induced lung tumorigenesis in mice. We analyzed the inhibitory effect of vitamin E on ornithine decarboxylase, cyclooxygenase and lipoxygenase activities at a promotion phase of lung tumorigenesis in mice. An increase in the ODC of urethane treated-mice and no significant change in the ODC of VE-treated mice were observed. An increase in the production of PGE2 and all HETES tested in the lungs of the urethane-treated mice was observed at week 8 after injection (promotion phase), showing a significant difference compared to the control group. Excessive vitamin E feeding during the initiation or promotion phases inhibited the increase in PGE2 and HETES produced by urethane treatment. These results suggest that the suppression of prostaglandin metabolism and ODC may be associated with the inhibitory effect of vitamin E against urethane-induced lung tumorigenesis.

Key Words mouse lung tumorigenesis, vitamin E, ornithine decarboxylase, cyclooxygenase, lipoxygenase

We and others have reported that vitamin E is a useful chemopreventive agent to reduce lung tumorigenesis in humans and experimental animals (1–4). However, *To whom correspondence should be addressed.

Abbreviations: ADAM, 9-anthryldiazomethane; PGE2, prostaglandin E2; HETE, hydroxyeicosatetraenoic acid; ODC, ornithine decarboxylase.
the detailed mechanism related to the vitamin E inhibitory effect on lung tumorigenesis is still unclear at present. We have done some studies to clarify the above mechanism in detail. As a result, we have found that the inhibition of ornithine decarboxylase (ODC) induction, the rate-limiting enzyme in the polyamine-biosynthetic pathway, and subsequent cell proliferation during the process of urethane-induced lung tumorigenesis in mice contribute to the reduction of tumorigenesis by vitamin E (3).

Increased ODC activity and subsequent polyamine accumulation are accepted as biomarkers for tumor promotion in tumorigenesis (5). Also, the inhibition of ODC induction is closely related to the suppression of DNA synthesis and tumorigenesis (6). This report and our previous data lead us to hypothesize that vitamin E acts as a useful agent to reduce lung tumorigenesis through the inhibition of a signal pathway related to ODC induction. Prostaglandin inhibitors such as indomethacin can suppress the induction of ODC as well as lung tumorigenesis (7, 8). On the contrary, an increase of the prostaglandin E2 (PGE2) level is necessary to elevate ODC activity (9). Thus, the possibility exists that lowering prostaglandin-synthetic activity in the lung by vitamin E may reduce the development of lung tumors through to the inhibition of ODC induction. In this study, the possible inhibitory effect of vitamin E on pulmonary prostaglandin biosynthesis was investigated in mice treated with urethane.

Materials and methods

Animals, diets and feeding. Six week-old male, specific pathogen-free, ddY mice (SLC, Shizuoka, Japan) were used. The mice were fed a control CE-2 diet (vitamin E content, 20 mg/kg diet) or a vitamin E-supplemented CE-2 diet (vitamin E content, 400 mg/kg diet) (Clea Japan, Tokyo, Japan) and sterilized water ad libitum. Urethane (Sigma, St. Louis, MO, USA) was dissolved in saline. The mice were treated either with urethane solution (750 mg/kg, i.p.) or the vehicle on the first day of the experiment. Experimental groups were divided into the four groups shown in Fig. 1: control group (C), urethane-treated group (U), urethane + vitamin E-treated group 1 (E1) and urethane + vitamin E-treated group 2 (E2). The periods of excessive vitamin E feeding in the E1 and E2 groups were initiation and promotion phases, respectively. The period of vitamin E feeding during the initiation or promotion phase was determined according to our previous data on the time course of pulmonary ODC activity after urethane injection (unpublished data).

Measurement of ODC, lipoxygenase and cyclooxygenase. At week 8, all mice were sacrificed under anesthesia with pentobarbital. A 20% lung homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.2). Supernatant and microsomal fractions from the homogenate were isolated by differential centrifugation (10). The supernatant fraction was used for ODC activity and lipoxygenase and the microsome fraction for cyclooxygenase assays. ODC activity was measured by radioactive CO₂ liberated from L-(1-¹⁴C)ornithine (11). The assay mixture of ODC

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Fig. 1. Experimental design.

Experimental week

-1 0 1 8 (weeks)

Urethane or vehicle injection

Vitamin E feeding

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contained 0.4 mM L-(1-4C)ornithine (0.123 µCi) (Amersham International, Buckinghamshire, England), 0.18 mM pyridoxal-5′-phosphate, 0.25 mM EDTA, 2 mM dithiothreitol, 0.25 mM EDTA, 2 mM Tris-HCl (pH 7.5) and enzyme source in a final volume of 0.1 mL. Cyclooxygenase and lipoxygenase activities were determined by previously published methods (12). The reaction mixture for cyclooxygenase contained 50 mM phosphate buffer (pH 7.4), 30 µM arachidonic acid, 1 mM glutathione and 0.3 mg sample protein at a final volume of 0.2 mL. The reaction mixture for lipoxygenase consisted of 100 mM Tris-HCl (pH 7.4), 50 µM arachidonic acid, 2 mM CaCl2 and 0.3 mg sample protein at a final volume of 0.2 mL. The reaction was performed at 37°C, and started by the addition of arachidonic acid. After incubation for 15 min at 37°C, the pH of the reaction mixture was adjusted to 3.0 with 1 N HCl, and PGE2 and HETES were extracted with ethyl acetate. The extracted sample was passed through a SEP-PAK C18 column (Waters Associates, Milford, USA), and the methanol eluate was then evaporated (13). To determine cyclooxygenase activity, the PGE2 content in the residue was estimated using an ELISA system (Calbiochem, CA, USA). In the case of lipoxygenase activity determination, HETES in the residue were converted to their ADAM-derivatives based on the reaction with ADAM ethyl acetate. After fractionation through a cartridge of SEP-PAK Silica (Waters Associates), the levels of ADAM derivatives of HETES in the fraction were determined using a HPLC system equipped with an ODS-column and fluorescence detector (14).
Statistical analysis. Statistical comparisons were performed by Student’s or Welch’s t-test after analysis of variance. \( p < 0.05 \) was used for significant difference.

Results and discussion

As shown in Fig. 2, the increased levels of pulmonary lipoxygenase and cyclooxygenase activity in mice as the result of urethane treatment were suppressed by excessive vitamin E feeding during the initiation (E1 group) or promotion (E2 group) phases of lung tumorigenesis. The blood \( \alpha \)-tocopherol contents (values are average of two rats) of each group were 1.2 \( \mu \)g/mL (C group), 1.1 \( \mu \)g/mL (U group), 1.7 \( \mu \)g/mL (E1 group) and 2.1 \( \mu \)g/mL (E2 group). The administration of urethane significantly elevated the levels of PGE2 and HETES in the lungs as compared to the control group. The feeding of vitamin E significantly inhibited pulmonary 5-, 12- and 15-HETES formation (30–60% inhibition), as compared to the urethane-treated group. Also, PGE2 production showed a similar tendency to HETES formation. The induction of tumor promotion in some organs by several carcinogens is believed to be governed by metabolites such as PGE2 and HETES.

Fig. 2. The effect of vitamin E on pulmonary PGE2, 5-HETE, 12-HETE and 15-HETE production. Each column represents the mean from five determinations, and vertical lines indicate SEM. *Significantly different from control (C); †significantly different from urethane-treated group (U); ‡significantly different from urethane + vitamin E group (E1). C, control group; U, urethane-treated group; E1, urethane + vitamin E-treated group; E2, urethane + vitamin E-treated group.
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Fig. 3. The effect of vitamin E on ornithine decarboxylase activity. Each column represents the mean from five determinations and vertical lines indicate SEM. *Significantly different from control group (C); # significantly different from urethane-treated group (U). C, control group; U, urethane-treated group; E1, urethan + vitamin E-treated group; E2, urethane + vitamin E-treated group.

Such an assumption is strongly supported by studies showing that inhibitors of these metabolites suppress tumor growth (16). Therefore, the inhibitory effect of vitamin E on pulmonary lipoxygenase and cyclooxygenase observed in this study may contribute to the preventive effect of the vitamin against lung tumorigenesis. Additionally, vitamin E feeding at both phases (initiation and promotion) of lung tumorigenesis similarly inhibited the metabolism of arachidonic acid. This result indicates that vitamin E acts as a strong chemopreventive agent at both the initiation and promotion phases of lung tumorigenesis.

It is known that the induction of ODC activity is observed during carcinogenesis and that the induction of ODC activity precedes cell proliferation in many cells (17). As shown in Fig. 3, the ODC activity in the lungs of mice of the U group was increased, while that of the E1 and E2 groups returned to the level of the C group. This may indicate that the inhibitory effect of vitamin E on carcinogenesis leads to a decrease in ODC activity. With regard to the contribution of lipoxygenase- and cyclooxygenase-produced metabolites of arachidonic acid to lung tumorigenesis, the detailed mechanism is unclear at present. However, the role of PGE2 in lung tumorigenesis was partly clarified. In the A549 lung adenocarcinoma cell line, the cells spontaneously released PGE2 into the medium during proliferation, and the prostaglandin produced could act as a growth factor for the cells (18). Since PGE2 might stimulate ODC induction and subsequent cell proliferation through the activation of PGE receptor and related signal transduction (19), the use of vitamin E to inhibit PGE2 production during lung tumorigenesis is also considered as mechanism of vitamin-related suppression against tumorigenesis.

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