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SHORT COMMUNICATION

The Modulation Effect of Vitamin E on Prostaglandin E_2 Level and Ornithine Decarboxylase Activity at the Promotion Phase of Lung Tumorigenesis in Mice

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ABSTRACT. The present study was undertaken to investigate a mechanism of the inhibitory effect of vitamin E in urethane-induced lung tumorigenesis in mice. We assayed ornithine decarboxylase (ODC) activity and the prostaglandin E_2 (PGE_2) level in lung at 8 weeks after urethane injection (promotion phase). Excessive vitamin E feeding or indomethacin treatment suppressed the urethane-induced increase in ODC activity, while exogenous PGE_2 overcame the effect of vitamin E on ODC activity. Furthermore, the amount of PGE_2 and the level of ODC activity were well correlated. These results indicate that the vitamin E-induced decrease in PGE_2 level probably contributes to the inhibition of ODC induction and the prevention of tumor development in the lung. BIOCHEM PHARMACOL 53;11:1757–1759, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. lung tumorigenesis, ornithine decarboxylase; prostaglandin E_2; vitamin E

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, the detailed mechanism related to the inhibitory effect of vitamin E on lung tumorigenesis is still unclear at present. In earlier attempts to clarify the above mechanism, we found that the suppression of abnormal cell proliferation during the process of urethane-induced lung tumorigenesis in mice by vitamin E contributed to the reduction of the tumorigenesis [3].

The increased activity of ornithine decarboxylase (ODC¶) and subsequent polyamine accumulation are accepted as biomarkers for tumor promotion in tumorigenesis [5]. In addition, the inhibition of ODC induction could be closely related to suppression of DNA synthesis and tumorigenesis. 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 synthesis inhibitors such as indomethacin (Ind) could suppress the induction of ODC as well as tumorigenesis in some tissues [6, 7]; on the contrary, the increase in prostaglandin E_2 (PGE_2) level was necessary to elevate ODC activity [8]. Thus, there exists the possibility that the lowering of PGE_2 production in the lung by vitamin E may reduce the development of lung tumors due to the inhibition of ODC induction. In this context, the present study was undertaken to clarify if vitamin E could inhibit the induction of ODC due to the decrease in PGE_2 production in the lung of mice treated with urethane.

MATERIALS AND METHODS

Six-week-old male, specific pathogen-free, ddY mice (SLC, Shizuoka, Japan) were used. The mice were fed control CE-2 diet (vitamin E content, 20 mg/kg diet) or special CE-2 diet (vitamin E content, 400 mg/kg diet) (Clea Japan, Tokyo, Japan). Sterilized water or a 0.002% water solution of Ind was given *ad libitum*. Urethane (Sigma, St. Louis, MO, USA) was dissolved in saline, and the mice were treated with urethane solution (750 mg/kg, i.p.). The mice in the PGE_2 treatment group were given the PGE_2 solution at a dose of 350 mg/kg body weight by a single subcutaneous injection at 4 hr before killing. The experimental animals were divided into five groups: control group, urethane-treated group, urethane vitamin E-treated group, urethane + Ind-treated group, and urethane + vitamin E + PGE_2-treated group. Starting at the age of 6 weeks, mice were fed the special CE-2 diet and given the Ind solution. On the eighth day after the start of feeding, each group was given urethane or vehicle. At 8 weeks after treatment, all

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¶ *Abbreviations:* PGE_2, prostaglandin E_2; ODC, ornithine decarboxylase; Ind, Indomethacin.

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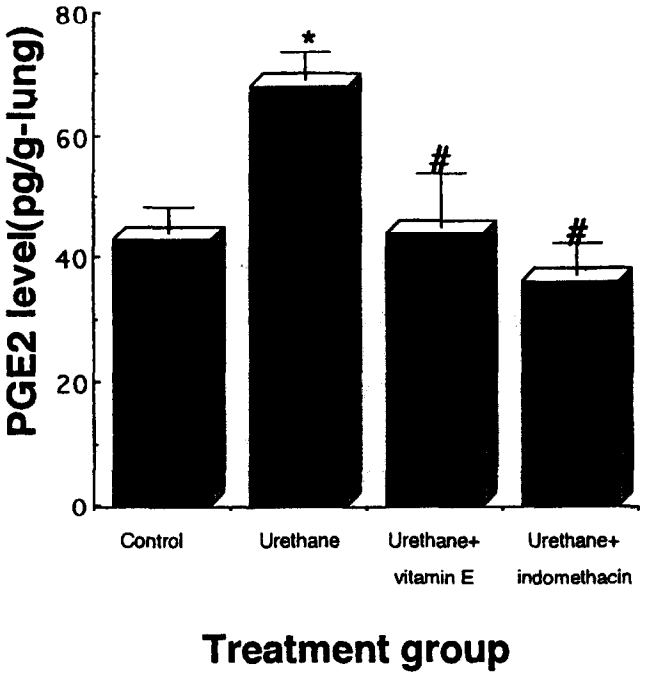

FIG. 1. The effect of vitamin E and indomethacin on pulmonary PGE_2 level in mice treated with urethane. Each column represents the mean from five determinations, and vertical lines indicate SEM. *Significantly different from control and #significantly different from urethane-treated group. Control, control group; Urethane, urethane-treated group; Urethane + vitamin E, urethane + vitamine E-treated group; and Urethane + indomethacin, urethane + indomethacin-treated group.

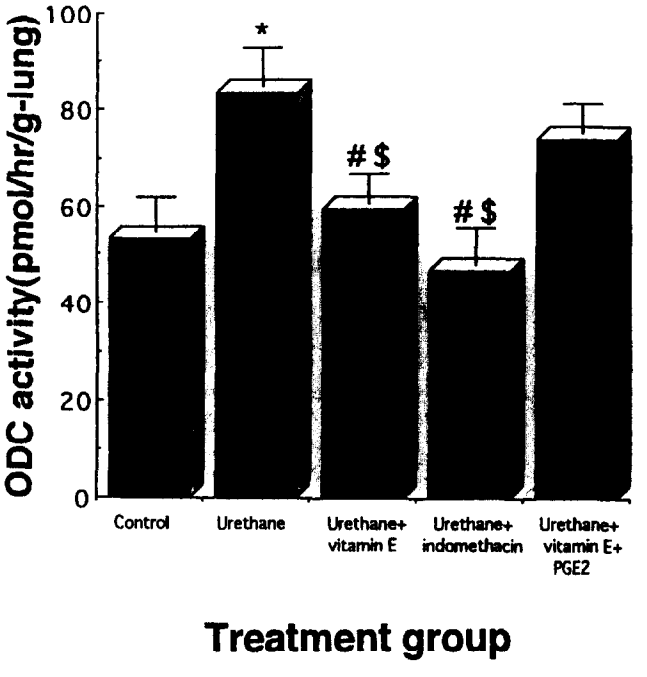

FIG. 2. The effect of vitamin E, indomethacin and PGE_2 on pulmonary ODC activity in mice treated with urethane. The assay mixture for ODC activity contained 0.4 mM L-[1-^{14}C]ornithine, 0.18 mM pyridoxal-5'-phosphate, 0.25 mM EDTA, 2 mM dithiothreitol, 25 mM sucrose, 50 mM Tris-HCl (pH 7.5), and 0.2 mg sample protein in a final volume of 0.1 mL, and the reaction was performed at 37°C. Each column represents the mean from five determinations, and vertical lines indicate SEM. *Significantly different from control, #significantly different from urethane-treated group, and §significantly different from urethane + vitamin E + PGE_2-treated group. Control, control group; Urethane, urethane-treated group; Urethane + vitamin E, urethane + vitamin E-treated group; Urethane + indomethacin, urethane + indomethacin-treated group; and urethane + vitamin E + PGE_2, urethane + vitamin E + PGE_2-treated group.

mice were killed under anesthesia with pentobarbital. A 20% lung homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4), containing 0.15 M sodium azide for the PGE_2 assay, and in 0.05 M Tris-0.25 M sucrose (pH 7.5) for the ODC assay. After centrifugation at 100,000 × g for 1 hr, the supernatant was acidified to pH 3.0 with 1 N HCl, and PGE_2 from the supernatant was extracted with ethyl-acetate. The extracted sample was passed through a SEP-PAK C_{18} column (Waters Associates, Milford, MA, USA), and the methanol eluate was then evaporated [9]. PGE_2 content in the residue was estimated by using an ELISA system (Cayman Chemical, Ann Arbor, MI, USA). In the ODC assay, the supernatant was used as enzyme source for ODC activity. ODC activity was determined by measuring the amount of radioactive CO_2 liberated from L-[1-^{14}C]-ornithine (Amersham, Buckinghamshire, UK) [10]. Statistical comparisons were performed by Duncan's multiple-range test after analysis of variance. $P < 0.05$ was used for significant difference.

RESULTS AND DISCUSSION

The effects of vitamin E and Ind treatment on PGE_2 level and ODC activity in the lungs of mice treated with urethane are shown in Figs. 1 and 2, respectively. The level of pulmonary PGE_2 in the urethane-treated group showed a 59% increase, significantly higher than that of the control group. Vitamin E and Ind treatment caused the level in the urethane-treated group to decrease by 35 and 47%, respectively. Furthermore, the differences in ODC activity among the groups were almost the same as differences in the PGE_2 level. Thus, there was a positive correlation between PGE_2 level and ODC activity in the lungs of mice ($r = 0.94$, data not shown). Moreover, PGE_2 injection restored 70% of the decreased portion of ODC activity observed in the urethane + vitamin E-treated group (Fig. 2).

The abnormal induction of ODC activity and the subsequent polyamine accumulation constitute an obligatory event in the promotion stage of experimental tumorigenesis [11]. In addition, the induction of ODC in mouse skin carcinogenesis has been demonstrated to be dependent upon production of prostaglandins, especially PGE_2 [12]. Thus, it seems that tumor development can be suppressed through the inhibition of PGE_2 production and subsequent ODC induction. Such an assumption is strongly supported by studies showing that Ind, an inhibitor of cyclooxygenase, suppresses ODC induction and tumor growth [12, 13]. Because it has been reported that vitamin E acts as an

inhibitor of cyclooxygenase [14], we hypothesized that vitamin E inhibited ODC induction via the suppression of PGE_2 production during the tumorigenic process of lung. This hypothesis can be supported by the data described above.

Although the detailed mechanism of the inhibition of ODC induction by vitamin E is unclear at present, previous reports lead us to assume an inhibitory mechanism of the vitamin on the induction [15, 16]. An elevation of the cyclic AMP level acts as a signal mediating hepatic ODC induction by a tumor promoter [15]. Additionally, it is thought that PGE_2 contributes to various cellular events through the activation of PGE receptor and related signal transduction, including cyclic AMP [16]. Thus, vitamin E inhibition of PGE_2-related events during lung tumorigenesis is considered as a mechanism in the suppression of the vitamin against ODC induction and tumorigenesis. However, further study is needed to confirm this hypothesis.

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