A Redox-Inactive Derivative of Tocotrienol Suppresses Tumor Growth of Mesothelioma Cells in a Xenograft Model

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Malignant mesothelioma (MM) is an aggressive cancer with poor prognosis. We focused on the anticancer activity of tocotrienol (T3) and have reported that a new redox-inactive T3 derivative (6-O-carboxypropyl-α-tocotrienol; T3E) exerts stronger inhibitory effects on MM cell growth than that of T3 in vitro. Furthermore, we have revealed some mechanisms of T3E that are involved in anti-MM effects. However, the effect of T3E in vivo remains unclear. In this study, we compared the plasma concentrations of T3E to that of T3 using mice to clarify differences in pharmacokinetics. Blood was sequentially collected after oral administration of T3 or T3E, and plasma concentrations were analyzed by HPLC. The area under the plasma T3 and T3E concentration–time curve from 0 to 24 h (AUC0–24h) of T3E was two times higher than that of T3. In addition, we evaluated the effect of T3E oral administration on tumor growth using a xenograft model of mice that were transplanted with human MM cells (H2052 cell line). Tumor volume was significantly reduced without body weight loss in mice orally administered 150 mg/kg T3E once per 2 d for 10 d, which suggests that T3E has potential anti-MM effects.

Key words tocotrienol derivative; plasma concentration; malignant mesothelioma

INTRODUCTION

Human malignant mesothelioma (MM) is an intractable cancer. The incidence of MM has been increasing worldwide because the onset of MM occurs long after exposure to asbestos fibers. In addition, the mortality is high because of the difficulty of diagnosis at an early stage, rapid progression, high invasion, and lack of effective treatments, and the median overall survival is about 1 year.1) Therefore, new innovative therapeutic approaches to MM are needed.

Tocotrienol (T3), a member of the vitamin E family, has anticancer activity. Although vitamin E is a strong antioxidant, its anticancer activity may be independent on its antioxidant activity. T3 has a short elimination half-life in vivo because of its high metabolic rate and antioxidant properties. Therefore, we synthesized a new succinate ether derivative of T3 (6-O-carboxypropyl-α-tocotrienol: T3E) from T3 to reinforce its anticancer effects (Fig. 1). Our previous in vitro studies have demonstrated that T3E has strong anti-MM effects compared with that of T3 and cisplatin using several MM cell lines without any toxicity for normal mesothelial cells.3) The anti-MM effect of T3E is mainly related to cap-dependent protein translation via rat sarcoma (RAS) and epigenetic modifications of a Wnt signaling antagonist in vitro.4,5) However, the differences between T3 and T3E on pharmacokinetics, such as blood concentration, and whether T3E administration suppresses MM cell growth in vivo are unclear. Here, we examined T3E as a new anti-MM drug candidate in mice.

MATERIALS AND METHODS

Reagents α-T3 was purchased from Tama Biochemical Co., Ltd. (Tokyo, Japan). T3E was synthesized from α-T3 according to a previously reported procedure.6) T3E purity was confirmed by GC-MS, 1H-NMR, 13C-NMR, and IR. NMR and IR spectra were consistent with the structure of T3E.1) 1H-NMR (CDCl3) spectrum: 1.27 (3H, s), 1.59 (9H, s), 1.67 (3H, s), 2.00 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 1.70–2.15 (16H, m), 2.57 (2H, t, J = 7.8 Hz), 2.65 (2H, t, J = 6.5 Hz), and 8.5 (1H, broad). IR (KBr) spectrum: 3200–3400 cm⁻¹ (carboxylic OH) and 1710 cm⁻¹ (C=O).

Cell Culture A human malignant pleural mesothelioma cell line H2052 was obtained from ATCC (Manassas, VA, U.S.A.). H2052 cells were grown in RPMI1640 (Thermo Fisher Scientific, Waltham, MA, U.S.A.) supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 50 µg/mL streptomycin (Nacalai Tesque, Kyoto, Japan) at 37°C in a humidified atmosphere with 5% CO₂.

Animals Six-week-old male ICR mice were obtained from Japan SLC Inc. (Hamamatsu, Japan) and habituated for 1 week before experimentation. Mice were housed under controlled light (12h light/dark cycle), temperature (24 ± 1°C), and humidity (55 ± 5%) conditions with MF food (Oriental Yeast Co., Ltd., Tokyo, Japan) and water available ad libitum. For the pharmacokinetic study, mice (n = 4) were fed by oral administration with T3 or T3E dissolved in tocopherol-stripped corn oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at a dose of 100 mg/kg. After administration, blood samples from T3 and T3E groups were collected by heparinized capillary tubes sequentially (1, 3, 6, 12, and 24 h).
separated by centrifugation of blood at 1000 × g for 20 min (4°C), and the supernatant was stored at −80°C.

Four-week-old male BALB/c-nu nude mice were purchased from Charles River Laboratories Japan Inc. (Yokohama, Japan). H2052 cells (5 × 10⁶ cells/mouse) suspended in phosphate buffered saline (PBS) with matrigel (1:1) were injected into the subcutaneous tissue on the backs of mice. After 1 week, mice were divided into 2 groups as equally average of tumor size, and received oral treatment of 150 mg/kg T3E or tocopherol-stripped corn oil (control) once per 2d for 10d. Then, the mice were weighed, and the tumor size was measured. Tumor volume was calculated as 1/2 × tumor size, and received oral treatment of 150 mg/kg T3E or tocopherol-stripped corn oil (control) once per 2d for 10d. Then, the mice were weighed, and the tumor size was measured. Tumor volume was calculated as 1/2 × large diameter × small diameter². At the endpoint, tumors were carefully removed after euthanization, and tumor weight was measured.

All animal experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee.

Plasma Concentration Analysis Sample preparation was performed based on the method of Ikeda et al. with modifications. An internal standard of 90 μg Tocol (Tama Biological Co., Ltd.), 350 μL 0.2% acetic acid, and 700 μL ethanol were added to 50 μL plasma. After sonication treatment by an ultrasonic homogenizer (Microtec Co., Ltd., Funabashi, Japan) for 45 s, 5 mL hexane was added to the mixture, vortexed for 1.5 min, and shaken for 20 min at room temperature. After centrifugation (1000 rpm, 4°C, 1 min), the supernatant was transferred to a glass test tube, and the hexane was vaporized by N₂ spray on the heat block at 40°C. The residue was dissolved in 100 μL ethanol and filtered using Ultrafree-MC-HV 0.45 μm (Merck, Darmstadt, Germany). An aliquot of 20 μL was injected into the HPLC system. The pump (EP-700, Eicom, Kyoto, Japan) was run at a flow rate of 700 μL/min, and the mobile phase was composed of a mixture of methanol–water–acetic acid (440:59:1). Chromatographic separation was performed by a COSMOSIL Packed Column 5PFP 4.6 mm i.d. × 250 mm (Nacalai) with a pre-column PC-04 filled AC-ODS (Eicom). The elution was monitored for peaks by a fluorescence detector (FP-2025Plus, Jasco, Tokyo, Japan) set at an excitation wavelength of 298 nm and an emission wavelength of 325 nm. Concentrations of T3 and T3E in the samples were determined by interpolation from the standard curve regression lines. The area under the plasma T3 and T3E concentration–time curve from 0 to 24 h (AUC₀–2₄h) was calculated using the trapezoidal rule.

Statistical Analysis Differences between groups were analyzed using a Student’s t-test. p Values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

Comparison of T3 and T3E Levels in the Plasma of Mice

Time-dependent changes of T3 and T3E levels in the plasma after the oral administration of a single 100 mg/kg dose to mice are shown in Fig. 2A. The peak plasma concentrations (Cmax) of T3 and T3E were 22.2 ± 5.3 and 19.5 ± 3.5 μmol/L, and the time required to reach Cmax (Tmax) was 3 and 6 h, respectively. The Tmax of T3 is approximately 3 h after oral administration in rats. Dietary vitamin E is secreted into the blood and lymph with chylomicron after absorption in the small intestine. However, there are differences between vitamin E homologues because of several factors, such as affinity for hepatic α-tocopherol transfer protein, transporter function in intestinal cells, and degradation by CYP.

In this study, the Tmax of T3E was delayed compared with that of T3, which may be related to ionization of the carboxyl group of T3E in the intestinal environment. Neuzil et al. has indicated that α-tocopheryl succinate (α-TOS), which is synthesized from tocopherol (another vitamin E homologue) and has a similar structure with T3E to be bonded succinate, will be charged [COO⁻⁻] at rate of 99% at neutral pH, while the proportion of its uncharged form [COOH] will increase at low pH (pH 6.2). It is thought that T3E is a weak acid with a similar pH with α-TOS (pKa of 5.64), and the charging of carboxyl group would influence for the different absorption rate between T3E and T3.
and T3 in the gastrointestinal system in mice. In addition, T3E had about two times higher AUC than that of T3 (Fig. 2B). We tested resistance to CYP using liver microsomes in vitro, and degradation of T3E was delayed compared with that of T3 (data not shown). Therefore, CYP affinity was altered by T3 derivatization. The improved bioavailability of T3E may be useful to utilize the side chain structure on T3. Interestingly, T3 was not detected after administration of T3E. Further investigation of the tissue distribution and metabolism of T3E is needed. 

**Anti-tumor Effect of T3E**

The effect of the oral administration of T3E on tumor volume, tumor weight, and body weight in mice were measured. Tumor volume was significantly reduced (Fig. 3A) and tumor weight was decreased (Fig. 3B) in the T3E treatment group while body weight was not affected (Fig. 3C). These results suggest that T3E has an anti-tumor effect with less toxicity in mice. Previously, we revealed that T3E has inhibitory effects on MM cell proliferation without influencing the viability of non-tumorigenic mesothelial cells, and T3 has no effects on either cell type.3) Furthermore, we found that Yes, a member of the Src kinase family, is a central mediator of MM cell growth,13) and T3E inhibits vascular endothelial growth factor (VEGF) expression via Yes signaling in the MM cell line H2452.14) T3E has similar effects in the H2052 cell line, and a comprehensive analysis of gene expression found that T3E modifies malignant factors such as RAS.4,5) These studies support the results of this in vivo study. In a similar report, α-tocopherylxybutyric acid, a non-antioxidative ether derivative of tocopherol, inhibits cell proliferation during the tumorigenic process of lungs in mice treated with tobacco-specific nitrosamines.15) Moreover, TOS reduces tumor volume targeted mitochondria in a mouse mesothelioma xenograft model.16) It is reported that T3 is a superior anti-cancer agent compared with that of tocopherol.17) Further studies including a toxicity test by administration of T3E for a long period are needed before clinical use. In conclusion, T3E is a new agent to treat MM based on the non-antioxidant functions of T3.

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**Conflict of Interest**
The authors declare no conflict of interest.

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