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Nutrition 25 (2009) 555–566

NUTRITION

www.nutritionjrnl.com

Basic nutritional investigation

Inhibition of proliferation and induction of apoptosis by γ-tocotrienol in human colon carcinoma HT-29 cells

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Manuscript received June 13, 2008; accepted October 17, 2008.

Abstract

Objective: γ-Tocotrienol is a major component of the tocotrienol-rich fraction of palm oil, but there is limited evidence that it has antitumor activity. In particular, the effects of γ-tocotrienol on human colon carcinoma cells have not been reported. To investigate the chemopreventive effects of γ-tocotrienol on colon cancer, we examined its capacity to inhibit proliferation and induce apoptosis in HT-29 cells and explored the mechanism underlying these effects.

Methods: We cultured HT-29 cells in the presence of γ-tocotrienol. The effect of γ-tocotrienol on cell proliferation was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, mitotic index, and colony formation. The cell-cycle distribution was investigated by flow cytometry. We measured apoptosis by nuclear staining, transmission electron microscopy, and DNA fragmentation. Apoptosis-related proteins and the nuclear factor-κB p65 protein were determined by western blotting and immunofluorescence.

Results: γ-Tocotrienol inhibited cell growth and arrested HT-29 cells in G_0/G_1 phase. The 50% inhibitory concentration was 31.7 μmol/L (48 h). γ-Tocotrienol–induced apoptosis in HT-29 cells was accompanied by downregulation of Bcl-2, upregulation of Bax, and activation of caspase-3. Furthermore, we found that γ-tocotrienol reduced the expression level of total nuclear factor-κB p65 protein and inhibited its nuclear translocation.

Conclusion: The results indicated that γ-tocotrienol inhibits cell proliferation and induces apoptosis in HT-29 cells in a time- and dose-dependent manner, and that this process is accompanied by cell-cycle arrest at G_0/G_1, an increased Bax/Bcl-2 ratio, and activation of caspase-3. Our data also indicated that nuclear factor-κB p65 protein may be involved in these effects.

Keywords: γ-Tocotrienol; Cell proliferation; Cell cycle; Apoptosis; Colon cancer cells

Introduction

Tocotrienols and tocopherols are two subclasses of vitamin E compounds that are abundant in food ingredients such as palm oil, rice bran oil, barley, corn, oats, rye, and wheat [1,2]. Dietary intake of palm oil, the richest known source of natural tocotrienols and tocopherols [3,4], suppresses chemically induced mammary tumorigenesis in female rats [5,6]. Furthermore, the tocotrienol-rich fraction (TRF) from palm oil (36% γ-tocotrienol, 18% α-tocotrienol, 12% δ-tocotrienol, and 22% α-tocopherol) [7] also has anticarcinogenic effects on human colon carcinoma [8] and prostate cancer [9] cells in vitro. The composition of TRF is approximately 75% tocotrienols and 25% α-tocopherol, but previous studies have demonstrated that its antiproliferative effects are mediated by tocotrienols, not α-tocopherol [10–12]. It is not completely understood why tocotrienols are more potent than tocopherols, but greater cellular accumulation is at least part of the reason [13]. Natural tocotrienols

This project was supported by the National Natural Science Foundation of China (grant 30471444).

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0899-9007/09/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2008.10.019

exist in four different forms or isomers, i.e., α-, β-, γ-, and δ-tocotrienol, which contain different numbers of methyl groups on the chromanol ring. Although all the isomers are effective antioxidants because a hydrogen atom from the hydroxyl group on the chromanol ring can readily be donated to reduce free radicals, each has its own biological activity. In particular, γ-tocotrienol is one of the most abundant forms of tocotrienol in foods [7]. Furthermore, various studies have indicated that γ-tocotrienol has significant anticancer activity [14–17]. In vivo, dietary γ-tocotrienol suppressed murine melanoma growth and increased host survival time [7]. In vitro, γ-tocotrienol proved cytotoxic to various human tumor cell lines [18–20] but had no toxic effect on the proliferation of normal cells [21,22]. For instance, it inhibits proliferation and induces apoptosis in MDA-MB-231 [17] and Hep3B [19] cells. Recent results from our laboratory have demonstrated that γ-tocotrienol induces apoptosis and metastasis in the human gastric adenocarcinoma cell line SGC-7901 by downregulation of the extracellular signal-regulated kinase signaling pathway [14,20]. However, details of the mechanism by which γ-tocotrienol inhibits proliferation and induces apoptosis in tumor cell lines remain unclear.

Colon carcinoma is a serious health problem and one of the leading causes of cancer mortality worldwide, especially in developed countries [23]. Chemoprevention is a major strategy in cancer prevention, because therapies have not proved effective to date in controlling the high incidence or the low survival rate of human colon carcinoma [24]. Dietary factors that inhibit cell proliferation are an exciting prospect for cancer prevention and treatment. A 4-y study provided convincing data that a high vitamin E intake was associated with a reduced risk of colon carcinoma, particularly for women younger than 65 y [25]. It has been reported recently that the TRF has antiproliferative effects and induces apoptosis in human colon carcinoma RKO cells [8]. However, the effects of individual TRF components on human colon carcinoma cell proliferation and the possible mechanisms involved remain unclear. γ-Tocotrienol has potent biological and pharmacologic activities. The objectives of our present study were to evaluate the effects of γ-tocotrienol on proliferation and apoptosis in HT-29 cells and to investigate the underlying molecular mechanism. Our results suggest that a possible molecular mechanism involves the suppression of nuclear factor-κB (NF-κB) p65 protein expression and its nuclear translocation, resulting in a direct effect on cell-cycle progression and activation of the proapoptotic pathway.

Materials and methods

Materials

The human colon carcinoma HT-29 cell line was obtained from the Cancer Institute of the Chinese Academy of Medical Science. The Cycle Test PLUS DNA reagent kit was bought from Becton-Dickinson (Franklin Lanes, NJ, USA). γ-Tocotrienol was from Davos (Singapore). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethylsulfoxide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Giemsa stain was purchased from Amresco (USA). Rabbit polyclonal antibodies for β-actin (sc-1616-R), Bcl-2 (sc-492), caspase-3 (sc-7148), and NF-κB p65 (sc-372) were bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal antibody for Bax (sc-7480) was obtained from Santa Cruz Biotechnology. Goat anti-rabbit (w3960) and anti-mouse (w3950) secondary antibodies were purchased from Promega (Madison, WI, USA). Fluorescein isothiocyanate–conjugated fluorescent secondary antibody was purchased from Santa Cruz Biotechnology.

Cell culture

Human colon carcinoma HT-29 cells were maintained in RPMI-1640 medium (Gibco, Paisley, Scotland) in 75-cm2 flasks at 37°C in a 5% CO_2 atmosphere at constant humidity. The medium was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco), 2 mmol/L of L-glutamine (Gibco), and 1% antibiotic solution (Gibco) and was changed every other day. The same dose of ethanol vehicle was used in the control cell culture. For subculturing, cells were rinsed once with phosphate buffered saline (PBS) and incubated in 0.25% trypsin containing 0.02% ethylene-diaminetetra-acetic acid (EDTA; Gibco) in PBS for 3 min. For the γ-tocotrienol supplementation experiment, stock solutions of γ-tocotrienol were prepared in absolute ethanol and stored at −20°C. The final ethanol concentration in all cultures was 0.15% [15].

Cell viability

The effect of γ-tocotrienol on cell viability was determined by an MTT assay. Briefly, cells were seeded in 96-well microtiter plates (Nunc, Wiesbaden, Germany) at 1.0×10^4 per well. After 24 h of incubation, the medium was removed and the cells were treated with 200 μL of medium containing various concentrations (15, 30, 45, and 60 μmol/L) of γ-tocotrienol for the desired time. Control cells were supplemented with 0.15% ethanol vehicle. Each concentration of γ-tocotrienol was repeated in five wells. Twenty microliters of MTT (5 mg/mL in PBS) was added to each well and incubated at 37°C for 4 h. The medium was carefully removed and 150 μL of dimethylsulfoxide was added to each well. The plates were shaken for 10 min and the absorbance at 490 nm was measured in an Elx800 Universal microplate reader (Bio-Tek Instruments, Inc., USA). Growth inhibition by γ-tocotrienol was calculated as percentage of cell viability, taking the viability of the vehicle-treated cells as 100%.