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Induction of Quinone Reductase, an Anticarcinogenic Marker Enzyme, by Vitamin E in Both Hepa1c1c7 Cells and Mice

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Abstract

Induction of NAD(P)H:(quinone-acceptor) oxidoreductase (QR) which promotes obligatory two electron reduction of quinones and prevents their participation in oxidative cycling and thereby the depletion of intracellular glutathione, has been used as a marker for chemopreventive agents. We postulated that vitamin E, an antioxidant, which induces QR as the gene of QR was reported to contain antioxidant responsive element in the 5'-flanking region. Vitamin E resulted in significant induction of QR in both hepa1c1c7 cells and mouse tissues. QR induction was observed to be maximal at 25 μM vitamin E for hepa1c1c7 cells while it was maximal in the level of 2.5~5 μmoles vitamin E/kg BW for mouse tissues. Induction of arylhydrocarbon hydroxylase, one of phase 1 enzymes, showed similar pattern to QR induction in mouse tissues. Thus the cancer-preventive effect of vitamin E may be exerted by its induction of intracellular detoxifying enzymes.

Key words: vitamin E, quinone reductase, hepa1c1c7 cells

INTRODUCTION

Induction of phase 2 enzymes such as quinone reductase (QR; NAD(P)H:(quinone-acceptor) oxidoreductase (EC1.6.99.2)), and glutathione S-transferase(GST) is closely associated with the prevention of chemical carcinogenesis (1). Accordingly screening of quinone reductase inducers from the natural products has drawn much attention and diverse chemical agents such as oxidizable diphenols, isothiocyanates, and even hydrogen peroxide were identified to induce quinone reductase (2). Majority of QR inducers are reported to be electrophiles and interact with antioxidant responsive element (ARE) of QR gene to increase QR gene expression. Many antioxidants are electrophiles and have a potential to induce phase 2 enzymes by interacting with ARE sequence on their genes (3). There is a good possibility that vitamin E, one of the strong antioxidants, induces QR and thereby exerts anticarcinogenic activity. This study was performed to determine whether vitamin E contains the ability to induce the anticarcinogenic enzyme activity in cultured cells and mice organs.

MATERIALS AND METHODS

Cell culture

Hepa1c1c7 cells were plated at density of 5×10^5 cells/plate in 10 ml of α-MEM supplemented with 10% charcoal-treated fetal bovine serum (Gibco-BRL, Gaitherburg, MD, USA). The plates were grown for 3~4 days in humidified incubator in 5% CO_2 at 37°C. Hepa1c1c7 cells were cultured for 48 hrs, followed by exposure to various concentrations of alpha-tocopherol (Sigma, St Louis, MO USA) for another 24 hrs.

Animals and diets

ICR mice weighing about 20 g were obtained from Dae-Han Laboratory Animal Reserarch Center (Seoul, Korea) at 7 weeks of age. Each animal was housed in a stainless cage in a room at a temperature of 24±2°C and 55% humidity with a 12 hr light-dark cycle. They were maintained on Chow (ICN, Costa Mesa, CA, USA) and tap water *ad libitum* for 2 weeks before the start of the experiment. Starting at the age of nine weeks, five groups of 8 mice each received AIN-76 powdered basal diet and treatment groups were orally intubated with 0.25, 1, 2.5, 5 μmoles alpha-tocopherol per kg body weight once a day for 7 days. At the end of experimental period, animals were sacrificed by drawing blood from heart after being anesthetized. Liver, lung, kidney, and small intestine were removed, frozen in liquid nitrogen and stored at -70°C until the enzyme assayed.

Enzyme assays

QR activity was measured by the method described by Benson et al. (4) with slight modification (5). Briefly, hepa1c1c7 cells were plated, grown, and exposed to different concentrations of alpha-tocopherol for 24 hr before cell harvest. The cells were washed with ice-cold 0.15 M KCl-10 mM potassium phosphate (pH 7.4), removed from the plates by scraping with rubber policeman, and disrupted for 5 sec using ultrasonic cell disrupter (50 W, Kontes, Vineland, NJ, USA). Meanwhile, tissues taken from mice were homogenized in Polytron homogenizer for 1 min. Cell and tissue homogenates were centrifuged at 9,000×g for 20 min. Supernatant was centrifuged at 105,000×g for 60 min and separated into

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cytosolic and microsomal fractions. Cytosolic and microsomal fractions were used for QR and AHH assays, respectively. QR activity was assayed by measuring the rate of oxidation of 2,6-dichlorophenolindophenol at 600 nm in the assay system containing 25 mM Tris-HCl (pH 7.4), 0.7 mg crystalline bovine serum albumin at pH 7.4, 0.01% Tween 20, 5 μM FAD, 0.2 mM NADH, 0 or 10 μM dicoumarol, and 200 μl cell extract in a final volume of 3.0 ml. AHH activity was assayed as described previously (6). The microsomal pellet was resuspended in 30% glycerol-0.25 M potassium phosphate buffer (pH7.25 for liver, pH 7.55 for nonhepatic tissues) by gentle homogenization to give a final protein concentration of 8~12 mg/ml. The 1 ml reaction mixture included 50 umoles of postassium phosphate buffer (pH 7.25 for liver, pH 7.55 for nonhepatic tissues), 0.36 μmoles of NADPH, 0.39 μmoles of NADH, 0.1 ml of microsomal homogenate, and 80 nmol of benzo(a)pyrene (in 40 μl of methanol). The mixture was shaken at 37°C, for 10 min for liver and 20 min for nonhepatic tissues, and the reaction was stopped with 4.25 ml of cold hexane-acetone (3.25:1.00). One milliliter aliquots of organic phase were added to 3.0 ml volumes of 1 N NaOH. The 3-hydroxy-benzo(a)pyrene was immediately determined spectrofluorometrically (Ex 396 nm, Em 522 nm) and relative AHH activity (%) was calculated. Protein concentrations were determined by the method of Lowry (7).

RESULTS AND DISCUSSION

Cellular quinone reductase activity significantly increased at the concentration of 10 and 25 μM alpha-tocopherol although its induction ratio tended to be lowered at higher concentrations maybe due to cytotoxicity (Fig. 1). This result is consistent with the previous report that beta-carotene, retinoids, vitamin C, and E induce quinone reductase in Colo205 colon cancer cells in dose-dependent manner (8).

The intubation of more than 1 umoles alpha-tocopherol/kg BW caused a slight reduction in mouse body weight for one week experimental period maybe due to toxic effect (Table 1).

Meanwhile alpha-tocopherol supplement led to increase in quinone reductase activities of murine kidney, liver and lung (Table 2). Again the increase of alpha-tocopherol over 2.5 μmoles/kg BW caused suppression of the enzyme induction

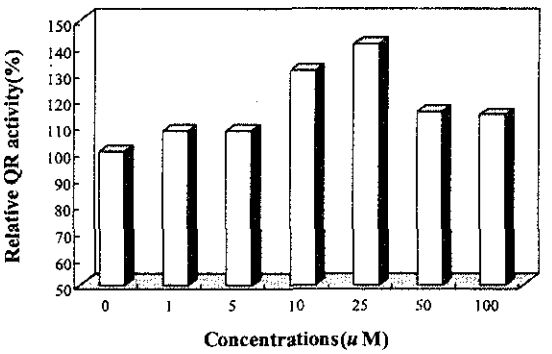

Fig. 1. Quinone reductase induction by vitamin E in hepa1c1c7 cells. 4×10^5 Cells were plated with 10ml alpha-MEM/10% FBS and incubated for 48 hrs and then exposed to various concentrations of alpha-tocopherol for 24 hrs, followed by measurement of QR activity.

as observed in cultured cells. Quinone reductase was induced more prominently in the whole animal system than *in vitro* cultured cells by the antioxidant nutrient, suggesting de novo generation is more active metabolite(s) than the parent compound in animal tissues such as liver.

Small proportion of ingested tocopherol has been proposed to be metabolized into 2,5,7,8-tetramethyl-2(2'-carboxyethyl) -6-hydroxychroman (alpha-CEHC), tocopherol quinone, and tocopherol hydroquinone (9). These compounds might have greater potential to induce QR activity than alpha-tocopherol itself.

As shown in Table 3, AHH activities in murine stomach and lung were induced by 3.0 to 4.7-fold when alpha-tocopherol was intubated at the concentration of 1.0 μmoles/kg BW. Overdose of alpha-tocopherol appeared to be ineffective in the induction of QR and AHH activities. As alpha-tocopherol induced phase I and II, the nutrient is assumed to be a bifunctional phase 2 enzyme inducer which causes QR induction mediated by cytosolic Ah receptor (5).

These results suggest that vitamin E might exert anticarcinogenic effect via modulation of detoxifying enzymes. In fact, several studies showed vitamin E protects animals from chemical carcinogenesis in liver, lung, colon, and stomach (10). However, there is no direct evidence supporting cancer protective effect of vitamin E on human subjects. In addition, the results of numerous trials on the preventive role of vita-

Table 1. Body and organ weights of mice fed different concentrations of alpha-tocopherol

Conc. of α-TE (μmoles/kg BW)	Body and organ weight (g)				
	Body	Liver	Kidney	Lung	Stomach
0	26.78 ± 0.79^{a}	1.26 ± 0.01^{ab}	0.41 ± 0.02^{a}	0.22 ± 0.04^{ab}	0.15 ± 0.01
0.25	26.15 ± 1.47^{a}	1.46 ± 0.11^{b}	0.38 ± 0.04^{ab}	0.32 ± 0.06^{b}	0.17 ± 0.01
1.0	21.25 ± 1.41^{b}	1.02 ± 0.02^{a}	0.33 ± 0.05^{ab}	0.16 ± 0.03^{a}	0.14 ± 0.01NS
2.5	22.37 ± 1.23^{b}	1.19 ± 0.02^{ab}	0.39 ± 0.02^{ab}	0.14 ± 0.02^{a}	0.14 ± 0.01
5.0	19.76 ± 0.70^{b}	1.39 ± 0.13^{b}	0.30 ± 0.03^{b}	0.19 ± 0.03^{a}	0.18 ± 0.03

Values are presented as mean±SE

Values with different superscript within same column are significantly different from each other ($p<0.05$) as evaluated by Duncan's multiple range test

NS represents no significant difference among treatment groups

Table 2. Quinone reductase induction by alpha-tocopherol in murine organs

Conc. of α-TE (μmoles/kg BW)	Quinone reductase activity (nmoles DCIP reduced/mg protein/min)				
	Kidney	Liver	Lung	Small intestine	Stomach
0	428.3±20.8[a]	56.4± 8.5[a]	130.0±12.3[ab]	902.0±30.3[ab]	8212.0±119.3[a]
0.25	606.0±35.1[c]	64.1± 9.1[a]	118.4± 8.5[a]	908.5±34.3[ab]	7585.4±464.9[a]
1.0	562.4±20.0[bc]	95.1± 8.5[bc]	155.8±11.6[bc]	908.7±42.7[ab]	8232.1±960.3[a]
2.5	512.1±60.4[abc]	73.2± 6.1[ab]	200.9±14.9[d]	992.9±50.7[b]	8384.6±795.8[a]
5.0	467.7±31.4[ab]	109.3±14.2[c]	169.7± 6.7[cd]	859.9±35.1[a]	7382.9±705.2[a]

Values are presented as mean±SE
Values with different superscript within same column are significantly different from each other ($p<0.05$) as evaluated by Duncan's multiple range test

Table 3. Effect of alpha-tocopherol on arylhydrocarbon hydroxylase activity in mouse organs

Conc. of α-TE (μmoles/kg BW)	Relative AHH activity				
	Kidney	Liver	Lung	Small intestine	Stomach
0	9.79±1.67	108.86±15.10[a]	6.65±0.84[a]	24.55±4.11	16.38± 1.67[a]
0.25	10.20±1.34	188.91±68.87[ab]	15.16±3.61[ab]	24.74±8.71	34.69±12.41[a]
1.0	9.22±2.02NS	311.01±87.38[ab]	19.97±5.27[b]	14.95±5.15NS	77.41±22.52[b]
2.5	7.92±1.09	225.95±70.85[ab]	10.73±2.30[a]	11.88±4.46	15.12± 2.50[a]
5.0	7.61±1.33	379.55±58.96[b]	7.33±0.93[a]	12.39±4.00	19.54± 2.11[a]

Values are presented as mean±SE
Values with different superscript within same column are significantly different from each other ($p<0.05$) as evaluated by Duncan's multiple range test
NS represents no significant difference among treatment groups

mins (e.g. beta-carotene, retinols, retinoids, alpha-tocopherol, ascorbic acid, folic acid) against cancer are contradictory and far from being conclusive (11). The recommendation of vitamin E supplement for cancer prevention should be reserved until its efficacy is confirmed through more extensive clinical and epidemiological studies.

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