Yonsei Medical Journal
Vol. 25, No. 1, 1984

Effect of Antioxidants on the Incidence of 7,12-dimethylbenzanthracene-induced Mammary Tumor in Rats*

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The inhibitory effect of selenium, vitamin E, and BHA on DMBA-induced mammary tumorigenesis in rats was investigated. Dietary vitamin E (200 IU/Kg diet) alone could not reduce the tumor incidence at 25 weeks after DMBA administration (10mg DMBA/rat) when selenium was deficient. Selenium supplementation (2ppm in drinking water) to rats fed a practical diet (0.17 ppm Se) reduced the tumor incidence to 14.3% from 75% at 27 weeks after DMBA administration. Dietary supplementation of BHA (0.75%) also reduced the incidence of DMBA-induced mammary tumor to 42.9% at 27 weeks after DMBA-treatment. Rats fed a diet deficient in both selenium and vitamin E contained significantly lower glutathione peroxidase activity and higher malondialdehyde in muscle. However, supplementation of selenium or BHA to the rats fed a practical diet did not alter the malondialdehyde content and glutathione peroxidase activities in muscle, skin and mammary gland. Dietary selenium increased the tissue selenium level.

DMBA-induced mammary tumorigenesis was reduced by antioxidants tested but the anticarcinogenic effect of selenium or BHA seems to be independent of glutathione peroxidase activity.

Key Words : Selenium, BHA, Vitamin E, DMBA, Mammary Tumor

The anticarcinogenic activity of antioxidants such as selenium, vitamin E and ascorbic acid has been reported and verified in a number of studies (Harman, 1969; Shamberger, 1970; Weisburger, 1979; Thompson and Becci, 1980; Soullier *et al.*, 1981; Jacobs, 1983). The protective effect of selenium against chemically-induced tumorigenesis in animals has been demonstrated by many investigators (Harr *et al.*, 1973; Griffin and Jacobs, 1977; Welsch *et al.*, 1981; Median *et al.*, 1983), and there is growing evidence that dietary vitamin E inhibits the occurrence of gastrointestinal cancer (Cook and McNamara, 1980; Shklar, 1982). Although the biochemical mechanism by which these antioxidants function in the prevention of tumorigenesis is unknown, it has been suggested that the development of tumor in animals was

Received May 8, 1984

* This Study was Supported by Faculty Research Fund (1981-1982), Yonsei University, College of Medicine

influenced somehow by unknown dietary factors. It has been reported that the occurrence of chemically induced mammary tumor in rats was affected by dietary fat (Ip and Sinha, 1981) and the development of mammary tumor in C_3H mice was reduced by selenium when standard chow diet was fed but no effect of selenium was observed when animals were fed a purified casein diet regardless of the kind of oil added to the diet (Whanger *et al.*, 1982). Therefore, an experiment was conducted in order to investigate the anticarcinogenic efficacy of various antioxidants and to define the possible relationship between the development of the tumor and lipid peroxidation. In the present study, mammary tumor was induced by 7, 12-dimethylbenz (α) anthracene (DMBA) administration in rats given different diets, and the effect of selenium, vitamin E and 2,3-tertbutyl-(4)-hydroxy-anisole (BHA) on the incidence of tumor was studied.

MATERIALS AND METHODS

Animals and Diets

In the first experiment, forty (fifty days old) female albino rats were divided into four groups (10 rats/group) and were fed a diet (Table 1) supplemented with selenium (0.2ppm Se as sodium selenite), vitamin E (200 IU/Kg diet) or BHA (0.75%). Food and water were available *ad libitum*. In the second experiment, 22 female albino rats (50 days old) were divided into 3 group; basal, +BHA (0.75% in the diet) and +Se (2ppm of sodium selenite in drinking water) groups. The basic diet was commercial calf pellet (0.17 ppm Se) with corn oil added (10% by weight). Animals were fed the respective diets, *ad libitum*, for 27 weeks.

Mammary Tumor Induction

Mammary tumors were induced by *i.g.* administration of 10 mg of DMBA (Sigma Chemical Co., St. Louis, U.S.A.) at 50 days of age. Rats were palpated weekly to determine the appearance of tumor and sacrificed at 25 weeks or 27 weeks after the DMBA treatment.

Table 1. Composition of basal diet

Ingredient	% of the diet
Torula yeast	30.0
Glucose	55.7
Vitamin E-free lard	5.0
Cod liver oil	3.0
Salt mixture	5.0
Vitamin mixture*	1.0
DL-methionine	0.3
Total	100.0

* Vitamin mixture(per 100g): Glucose 88.58g; Thiamin HCl, 40mg; Pyridoxin HCl, 20mg; Pantothenic acid, 200mg; Choline cholide, 10g; Niacine, 1g; Menadione, 10mg; Folic acid, 20mg; Biotin, 10mg; Vit. B_{12} (0.1% B_{12}), 100mg; Riboflavin, 25mg.

Measurement of Selenium, Malondialdehyde and Enzyme Activity

Selenium concentrations in muscle (pectoralis profundus), mammary gland and skin (abdomen) were measured by the method described previously (Oh and Cho, 1983). Malondialdehyde content in muscle, skin and mammary gland were measured by the method of Sinnhuder and Yu (1958), and glutathione peroxidase activity was measured according to the method of Paglia and Valentine (1967). Tissues (muscle, mammary gland, skin) were homogenized with 2 volumes of phosphate buffer (0.05M, pH 7.0) by the tissue homogenizer and the homogenates were centrifuged at 10,000 g for 15 min and the supernate fraction was used for the enzyme assay.

Selenium and malondialdehyde contents in the whole homogenate were measured. Protein was measured by the method of Lowry *et al.* (1951).

RESULTS

Experiment 1

Many rats fed a basal diet dificient in Se and vitamin E died before the appearance of solid tumor (Table 2). Supplementation of selenium and vitamin E could reduce the mortality of rats but the supplementation of vitamin E alone was not sufficient for the maintenance of healthy rats. BHA addition to the selenium supplemented diet could not reduce the mortality as in vitamin E supplementation. The incidence of mammary tumor at 25 weeks after DMBA administration was lowest in the group supplemented with both selenium and vitamin E and was highest in the group supplemented with no

Table 2. Effect of Se, vitamin E and BHA on the mortality and incidence of tumors in rats given DMBA

Group[b]	No. of rats	No. of dead or tumor bearing rats at following times (week) after DMBA administrtion.[a]													Total	Mortality (%)	Tumor incidence[c] (%)
		2	4	6	8	10	12	14	16	18	20	22	24	25			
+E, +Se	10	1	0	0	1	0	0	0	0	0	0	0	0	0	2	20	
		0	0	0	0	0	0	0	0	1	1	0	0	0	2		25
+E, -Se	10	2	0	0	0	0	0	0	0	0	0	1	0	1	4	40	
		0	0	0	1	0	0	0	0	0	0	2	0	0	3		50
-E, -Se	10	1	3	0	0	0	0	0	1	0	2	0	0	0	7	70	
		0	0	0	0	0	0	0	0	0	0	0	1	1	2		33
+BHA, +Se	10	0	0	0	1	0	1	0	1	0	1	0	0	0	4	40	
		0	0	0	0	0	0	0	0	0	0	1	0	0	2		33

a. DMBA (10mg/rat) was administered i.g. at the age of 50 days.
b. Vitamin E (200 IU/Kg), selenium (0.2 ppm), BHA (0.75%) were supplemented to the basal diet.
c. Tumor incidence (%) = $\frac{\text{No. rat bearing tumor}}{\text{No. rat survived at 25 weeks after DMBA treatment}} \times 100$

Table 3. Effect of dietary antioxidants on lipid peroxidation in muscle (pectoralis profundus) and mammary tissue of rats treated with DMBA

Group	Malondialdehyde[a]	
	Muscle	Mammary gland[b]
+E, +Se	5.23±1.20	13.5 (2)
+E, -Se	5.15±1.29	16.5 (2)
-E, -Se	29.20±1.48*	8.5 (1)
+BHA, +Se	6.17±0.85	17.6±4.6(3)

All values are mean ± S.D.
a. Malondialdehyde (nmoles/g of wet tissue)
b. Samples were taken from tumor mass and No. in parenthesis is No. of rat analyzed.
* $p<0.001$; Compared with +E, +Se group.

Table 4. Effect of dietary antioxidants on glutathione peroxidase activity in muscle (pectoralis profundus) and mammary gland of DMBA treated rats

Group	Glutathione peroxidase[a]	
	Muscle	Mammary gland[b]
+E, +Se	60.8±7.5	85.5 (2)
+E, -Se	10.3±4.1*	22.4 (2)
-E, -Se	8.9±2.7*	17.6 (1)
+BHA, +Se	63.7±9.9	100.5±11.7 (3)

All values are mean ± S.D.
a. Glutathione peroxidase (nmoles NADPH oxidized/min/mg protein
b. Samples were taken from tumor mass and number in parenthesis is No. of rat.
* $p<0.001$; Compared with +E, +Se group.

Table 5. Effect of selenium and butyrated hydroxyl anisole on the incidence of DMBA-induced tumor in rats.

Group[a]	No. of rats	No. of tumor brearing rats at following times (week) after DMBA administration[b]											Total	Tumor incidence (%)
		17	18	19	20	21	22	23	24	25	26	27		
Basal	8	0	0	0	0	1	0	0	1	0	3	1	6	75.0
+BHA(0.75%)	7	0	0	0	0	1	0	0	0	2	0	0	3	42.9
+Se (2 ppm)	7	0	0	0	0	1	0	0	0	0	0	0	1	14.3

a. Basal diet was made of calf-pellet plus 10% corn oil.
BHA was added to the basal diet and Se(Sodium selenite) was supplied in the drinking water ad libitum.
b. DMBA (10mg/rat) was given i.g. from the age of 50 days.

Table 6. Effect of BHA and selenium administration on selenium contents in DMBA-treated rat tissues

Group[a]	Selenium content (μg Se/g tissue)		
	Muscle (Pectoralis profundus)	Skin (Abdomen)	Mammary gland (Tumor mass)
Basal	0.27± 0.05	0.14±0.04	0.40±0.06
+BHA	0.25±0.06	0.13±0.01	0.36±0.10
+Se	0.32±0.05	0.49±0.12*	0.50 (1)

All values are mean ± s.D.
a. Basal diet was made of calf pellet (0.17 ppm Se) plus 10% corn oil by weight.
BHA (0.75%) was added to the basal diet and selenium (2 ppm) was supplemented into drinking water.
* Significant difference from basal group ($p<0.05$).

Table 7. Effect of BHA and selenium administration on malondialdehyde contents in tissues of rats treated with DMBA

Group[a]	Malondialdehyde (n moles/g tissue)		
	Muscle (Pectoralis profundus)	Skin (Abdomen)	Mammary gland (Tumor mass)
Basal	28.2 ± 6.7	12.3±4.7	8.5±3.5
+BHA	57.6±24.7	10.3±3.0	–
+Se	57.5±25.7	7.7±1.2	7.8 (1)

All values are mean ± S.D.
a. Basal diet consists of calf pellet plus 10% corn oil. BHA (0.75%) was supplemented to the basal diet and selenium was supplied in drinking water (2 ppm Se).

Table 8. Effect of BHA and selenium administration on glutathione peroxidase activity in tissues of rats treated with DMBA

Group[a]	Glutathione peroxidase (n moles of oxidized NADPH/min/mg of protein)		
	Muscle (Pectoralis profundus)	Skin (Abdomen)	Mammary gland (Tumor mass)
Basal	54.0±13.9	73.7± 4.1	105.5 ± 9.3
+BHA	39.4± 7.0	72.4±18.4	103.8±22.9
+Se	47.6± 7.3	78.8± 3.6	125.7 (1)

All values are mean ± S.D.
a. Basal diet consists of calf-pellet plus 10% corn oil. BHA (0.75%) was supplemented to the basal diet and Se (2 ppm) was supplied in drinking water.

selenium (Table 2). There was a significant increase in malondialdehyde content in muscle of rats fed with basal diet (Table 3), however, no significant differences in the malondialdehyde content in mammary gland were observed between groups. Glutathione peroxidase activity in the muscle of rats fed a diet supplemented with selenium was significantly higher than that of rats fed with no selenium (Table 4). Glutathione peroxidase activity in mammary gland was slightly higher than that of muscle, and the effect of selenium on the enzyme activity was similar to that in muscle (Table 4).

Experiment 2

Slelnium supplementation to drinking water

reduced the incidence of mammary tumor to 14.3% from 75% at 27 weeks after DMBA-treatment. Dietary supplementation of BHA also reduced the incidence of tumor to 42% from 75% (Table 5). The tumor appearance usually notified at 24 weeks after DMBA treatment. Selenium concentrations in muscle, mammary gland and skin were increased by selenium supplementation and the skin selenium level was significant (Table 6).

There were no differences in glutathione peroxidase activities in tissues between groups (Table 7), but relatively higher enzyme activities were observed in skin and mammary gland compared to those in muscle.

Muscle malondialdehyde level was generally higher than that in the skin or mammary tissue, although there were no significant differences in malondialdehyde content in tissues between groups (Table 8).

DISCUSSION

An inhibitory activity of short-term feeding of dietary selenium against the induction of mammary tumor by DMBA was studied using a Torula yeast-based low Se and low vitamin E diet (Thompson *et al.*, 1982). An attempt was made to keep rats deficient in selenium or vitamin E by feeding Torula yeast-based diet and this basal diet was then supplemented with selenium, vitamin E or BHA for the experimental study. Most of the rats fed a basal diet (deficient in both selenium and vitamin E) survived for only a short period of time, so it was difficult to evaluate the incidence of tumor by the small number of rats that survived. Rats started to develop palpable tumor at 20 weeks after DMBA treatment in this study. The period of time is somewhat longer than the period reported by Horvath and Ip (1983). In their study corn oil (20%) was used and they observed palpable tumors in 50% at 13 weeks of DMBA administration.

The discrepancy between these who results may be due to the fat content in the diet. This fact was confirmed in the report that a high level of dietary fat increases the rate of the appearance of breast cancer (Chan and Cohen, 1974). The present study showed that vitamin E was unable to inhibit the tumorigenesis of mammary gland by DMBA administration but it could potentiate the selenium effect of anti-tumorigenesis. The tissue level of malondialdehyde, a carcinogen itself (Shamberger *et al.*, 1974), was increased in rats deficient in both selenium and vitamin E but it has no correlation to mammary tumorigenesis in the present study.

BHA, a synthetic antioxidant widely used as a food additive, had an inhibitory effect on mammary tumor. Two possible mechanisms of anticarcinogenesis of BHA are considered. It may function as a free radical scavenger, or on the other hand, it may stimulate the induction of glutathione-S-transferase which is responsible for the metabolism of ultimate electrophilic carcinogen metabolites (Benson *et al.*, 1979; Oh and Lee, 1981). The reduction of tumor incidence by selenium supplementation to the drinking water, was remarkable in the present study (experiment 2). Several mechanisms of selenium inhibition of DMBA-induced mammary carcinogenesis are possible.

Selenium, as a component of glutathione peroxidase could prevent lipid peroxidation and thus protect against alterations in membrane structure (Hoekstra, 1975). Selenium inhibition of DMBA-carcinogenesis might also involve in DMBA detoxification by binding to active electrophilic carcinogen metabolites which are able to attack DNA or proteins. Another mechanism of tumor inhibition by selenium is the stimulation of the repair of carcinogen induced

DNA damage (Lawson and Birt, 1983) or the reduction of DNA alkylation which was shown in colon carcinogenesis (Harbach and Swenberg, 1981). Glutathione peroxidase activity in tissues of rats deficient in selenium (experiment 1) was significantly lower than that of selenium supplemented rats, however, there were no significant differences in enzyme activities between groups in experiment 2. It is evident that basal diet used in experiment 2 contains adequate amount of Se for the saturation of glutathione peroxidase in tissues (Oh *et al.*, 1976). The present study showed that selenium had the strongest antitumorigenic activity among the antioxidants tested but the inhibitory action of selenium in DMBA-induced mammary tumorigenesis seems to operate in some other mechanism besides the glutathione peroxidase system. It can not be excluded from the consideration that glutathione-S-transferase may be potentiated by selenium or induced by BHA, alleviates DMBA action in tumorigenesis.

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