

Modulation of 7,12-Dimethylbenz[*a*]anthracene-induced Transmammary Carcinogenesis by Disulfiram and Butylated Hydroxyanisole in Mice

Araga Ramesha Rao, Syed Perwez Hussain and Laxminarayana Jannu

Cancer Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

The individual as well as combined chemopreventive actions of disulfiram (DSF) and butylated hydroxyanisole (BHA) on 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced transmammary carcinogenesis in mice were examined. When nursing mothers receiving normal diet were treated with DMBA (1 mg/mouse) on days 6, 8 and 10 *postpartum*, the tumor incidence in their 50-week-old F₁ progeny was 44.1%. When nursing mothers receiving 0.75% BHA diet, 0.5% DSF diet and 0.75% BHA + 0.5% DSF diet were similarly treated with DMBA, the tumor incidences in their 50-week-old F₁ progeny were 14.7% ($P < 0.05$), 12.5% ($P < 0.05$) and 5.8% ($P < 0.01$), respectively. It is concluded that diets containing BHA (0.75%) and DSF (0.5%), singly or in combination, can inhibit transmammary carcinogenesis in Swiss albino mice.

Key words: 7,12-Dimethylbenz[*a*]anthracene — Transmammary carcinogenesis — Butylated hydroxyanisole — Disulfiram

When xenobiotic chemicals including carcinogens enter the body of female mammals it is likely that certain of these chemicals and/or their metabolites find their way into the offspring mainly through the transplacental (during gestation) and transmammary (during lactation) routes. Several workers have demonstrated that the exposure of pregnant animals to chemical carcinogens results in the occurrence of tumors in their progeny.¹⁻¹¹ Recently our laboratory showed that 7,12-dimethylbenz[*a*]anthracene administered to the P generation female mice could pass into the body of F₁ progeny through the transmammary route and produce tumors in them.¹²

Further, our laboratory has demonstrated that concomitant administration of butylated hydroxyanisole can inhibit transplacental as well as transmammary carcinogenesis induced by a single dose of DMBA in mice.^{7, 12}

This study was designed to assess the chemopreventive influences of BHA and disulfiram, given alone or in combination, on the transmammary carcinogenicity of multiple doses of DMBA in Swiss albino mice.

MATERIALS AND METHODS

Animals Inbred mice of Swiss albino strain were maintained in the air-conditioned animal facility and were provided (unless otherwise stated) with standard feed (Hindustan Lever Ltd., India) and tap water *ad libitum*. Females (10-12 weeks old) were allowed to mate with syngenic males of proven fertility and those with a vaginal plug (day '0' of gestation) were isolated and used for the experiments.

Chemicals 7,12-Dimethylbenz[*a*]anthracene (DMBA) was procured from Eastman Kodak Organic Chemicals,

Rochester, Minn.; Butylated hydroxyanisole (2(3)-*tert*-butyl-4-hydroxyanisole) (BHA) was procured from Sigma Chemical Co., St. Louis, Mo. while disulfiram (tetraethylthiuram disulfide) (DSF) was purchased from Aldrich Chemical Co., Milwaukee, Wis. DMBA was dissolved in peanut oil to give a concentration of 1 mg DMBA/0.1 ml oil, and administered by the intragastric route without anesthesia to the lactating mothers on days 6, 8 and 10 following parturition at the dose level of 1 mg DMBA/day/mouse. BHA and/or DSF were added to the powdered feed and thoroughly mixed in order to obtain 0.75% BHA diet, 0.5% DSF diet and 0.75% BHA + 0.5% DSF diet. These experimental diets were given to specific groups of nursing mothers (see Table I) during their lactation period for 21 days commencing from the date of parturition.

Experimental design The lactating mothers with their respective litters were assigned to control and experimental groups; each mother with her litter was kept in a separate cage. The following groups were maintained (see Table I): Group I: The nursing mothers ($n=6$) received 0.1 ml of peanut oil (vehicle for DMBA) on days 6, 8 and 10 *postpartum*. They were on normal diet during their entire lactation period. Group II: The nursing mothers ($n=6$) received 1 mg of DMBA on days 6, 8 and 10 *postpartum*. They were also on normal diet during their entire lactation period. Group III: The nursing mothers ($n=6$) received 1 mg of DMBA on days 6, 8 and 10 *postpartum*, and were on DSF diet during the entire period of lactation. Group IV: The nursing mothers ($n=6$) received 1 mg of DMBA on days 6, 8 and 10 *postpartum*, and were put on BHA diet during the entire period of lactation. Group V: The nursing mothers

(n=6) were treated with 1 mg of DMBA on days 6, 8 and 10 *postpartum*, and were put on DSF + BHA diet for the entire period of lactation. Groups VI–VIII: The nursing mothers (n=4) were each treated with the vehicle of DMBA (0.1 ml oil) on days 6, 8 and 10 *postpartum*, and were respectively put on DSF diet, BHA diet and DSF + BHA diet for the entire period of their lactation.

After weaning, the F₁ progeny of each group was separated by sex and kept for observation; normal diet and tap water were provided *ad libitum*. Body weight was recorded once in two weeks and palpable tumors, if any, were recorded at weekly intervals. All animals surviving at the age of 50 weeks were killed, and their tumors were harvested and classified histopathologically.

The data were analyzed statistically and the chi-square test was applied for calculating significance levels.

RESULTS

The findings of the present investigation are depicted in Table I. The body weight record of F₁ progeny (not presented in the table) showed no effects of transmammary exposure to the carcinogen and/or modulator(s) on weight gain during the observation period.

Animals of the control group (Group I) did not yield any tumor at the end of the 50-week observation period. When animals were exposed to DMBA by the transmammary route (Group II) 44.1% of them developed tumors. When F₁ progeny were exposed by the transmammary route to DMBA as well as DSF (Group III) the tumor incidence fell to 12.5%. When F₁ progeny were exposed by the transmammary route to DMBA and BHA (Group IV) the tumor incidence was 14.7%. The F₁ progeny showed only 5.8% tumor incidence when they were treated by the transmammary route with DMBA as well as BHA + DSF. When the F₁ progeny were exposed through the transmammary route only to the modulators (Groups VI, VII and VIII) no tumors developed in them.

The tumors developed in F₁ progeny following their transmammary exposure to DMBA were lung adenomas, mammary tumors, ovarian tumors, salivary gland tumors, lymphomas, skin papillomas, subcutaneous sarcomas and forestomach tumors (Table II).

DISCUSSION

In the present experimental model system of transmammary carcinogenesis both BHA and DSF exhibited chemopreventive action, singly as well as in combination.

Table I. Modulatory Influence of Disulfiram and Butylated Hydroxyanisole on DMBA-induced Transmammary Carcinogenesis in Mice

Groups	Treatment(s) of the nursing mothers during lactation period	No. of nursing mothers	No. of F ₁ progeny				F ₁ progeny with tumors		Total tumor incidence in F ₁ progeny (%)
			Initial		Effective		♂	♀	
			♂	♀	♂	♀			
I	Control (vehicle) Normal diet	6	21	17	18	16	0	0	0/34 (0)
II	DMBA: 1 mg × 3 days Normal diet	6	18	21	16	18	6	9	15/34 (44.1)
III	DMBA: 1 mg × 3 days DSF diet (0.5%)	6	20	16	17	15	2	2	4/32 ^{a)} (12.5)
IV	DMBA: 1 mg × 3 days BHA diet (0.75%)	6	19	18	17	17	2	3	5/34 ^{a)} (14.7)
V	DMBA: 1 mg × 3 days DSF (0.5%) + BHA (0.75%) diet	6	20	17	18	16	1	1	2/34 ^{b)} (5.8)
VI	Vehicle DSF diet (0.5%)	4	12	13	11	11	0	0	0/22 (0)
VII	Vehicle BHA diet (0.75%)	4	13	14	12	13	0	0	0/25 (0)
VIII	Vehicle BHA (0.75%) + DSF (0.5%) diet	4	12	14	11	12	0	0	0/23 (0)

a) P < 0.05 (group II vs. group III; group II vs. group IV).

b) P < 0.01 (group II vs. group V).

Table II. Tumor Types and Their Distribution in F₁ Male and Female Mice^{a)}

Group	Sex of F ₁ progeny	Tumors and their distribution in male and female mice								Total tumors
		Lung adenomas	Subcutaneous sarcomas	Mammary tumors ^{b)}	Ovarian tumors ^{c)}	Salivary tumors ^{d)}	Lymphomas	Skin papillomas	Forestomach tumors ^{e)}	
I	♂	0	0	0	0	0	0	0	0	0
	♀	0	0	0	0	0	0	0	0	0
II	♂	3	2	0	0	3	3	2	1	14
	♀	2	3	2	3	2	2	3	1	18
III	♂	1	0	0	0	0	1	0	0	2
	♀	0	0	1	1	0	1	0	0	3
IV	♂	1	0	0	0	1	0	1	0	3
	♀	2	0	2	1	0	1	0	0	6
V	♂	0	0	0	0	1	0	0	0	1
	♀	0	0	1	0	0	0	0	0	1
VI	♂	0	0	0	0	0	0	0	0	0
	♀	0	0	0	0	0	0	0	0	0
VII	♂	0	0	0	0	0	0	0	0	0
	♀	0	0	0	0	0	0	0	0	0
VIII	♂	0	0	0	0	0	0	0	0	0
	♀	0	0	0	0	0	0	0	0	0

a) For other particulars of the experiment, see Table I.

b) Fibroadenomas. c) Granulosa cell tumors.

d) Adenomas. e) Papillomas.

BHA has been shown to be an effective inhibitor of chemical carcinogenesis in single generation model systems.¹³⁻¹⁹⁾ Our laboratory has demonstrated the chemopreventive action of BHA in transplacental carcinogenesis induced by DMBA in mice.⁷⁾ Recent work in this laboratory which, however, differs from the present work as regards dose and administration schedule of DMBA also has shown that BHA can inhibit transplacental and transmammary carcinogenesis in mice.¹²⁾

Like BHA, DSF in the present study elicited a significant reduction in transmammary carcinogenesis in mice. DSF has already been shown to inhibit single generation carcinogenesis by several carcinogens.^{18, 20-22)}

Several mechanisms for the anticarcinogenic activities of BHA and DSF can be envisaged: 1) direct chemical interaction between the carcinogen (or its metabolites) and the modulators which results in neutralization of the reactive species; 2) direct suppression of the enzymatic activation of the carcinogen; 3) competition by the modulators (or their metabolites) for macromolecular binding sites of the ultimate carcinogen; 4) enhancement of mechanisms for the repair of damaged DNA; 5) stimulation of the immune system; and 6) induction of protective enzymes that inactivate the proximate or ultimate carcinogen. With respect to BHA, the possibility that

protection arises from an alteration in the metabolism of the carcinogen has received most attention. Mice consuming a diet containing BHA have markedly increased activities of glutathione *S*-transferase^{23, 24)} and UDP-glucuronyl-transferase.²⁵⁾ BHA has also been shown to inhibit the monooxygenase system²⁶⁾ and epoxide hydratase.^{25, 27-29)} In addition, BHA increases tissue glutathione levels.^{23, 25)} There are also studies to show that BHA can reduce the carcinogen-DNA adduct formation in mice.^{30, 31)} These properties of BHA are strongly suggestive that its chemopreventive action on chemical carcinogenesis is mediated through alterations in phase I and phase II reactions associated with drug detoxification. Besides, the role of BHA as a free radical scavenger in the inhibition of chemical carcinogenesis cannot be ruled out.

The mechanisms by which DSF, an antioxidant, protects against chemical carcinogenesis are less completely known. DSF can prevent chemical hepatotoxicity and lipid peroxidation in rats.^{32, 33)} Inhibition of monooxygenase by thionosulfur compounds has been reviewed by Fiala.³⁴⁾ Administration of DSF to mice has been shown to increase glutathione-*S*-transferase activity in the liver and the small intestine and also to increase the major hepatic glutathione transferase mRNA.^{1, 35-37)}

Induction of glutathione *S*-transferase and UDP-glucuronosyl-transferase activity in the liver by DSF and BHA prevents the toxic effect of diethylnitrosamine in rats.³⁸⁾ The anticarcinogenic and antimutagenic action of DSF was claimed to be due to the scavenging of reactive species.³⁹⁾

The combined chemopreventive action of BHA and DSF in the present transmammary carcinogenesis is additive in nature.

The present study suggests that transmammary carcinogenesis can be inhibited by potent chemopreventive agents given singly or in combination. Further experimental studies on this line should help us in tracking down other chemopreventive agents which might some day find application in the control of human perinatal carcinogenesis.

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