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Reduction in formation and growth of 1,2-dimethylhydrazine-induced aberrant crypt foci and tumors in the rat colon by resveratrol

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ABSTRACT: The purpose of the present study was to investigate the effect of resveratrol (RES) on 1,2-dimethylhydrazine (DMH)-induced rat colon carcinogenesis. The numbers of colon tumors and the formation of aberrant crypt foci (ACF), a premalignant lesion in rats were significantly reduced on resveratrol (8 mg/kg body weight) supplementation. Moreover, resveratrol supplementation for the entire treatment period showed significantly better chemopreventive activity against DMH-induced rat colon carcinogenesis as compared to resveratrol supplementation during the initiation and post-initiation stages of carcinogenesis.

KEYWORDS: Resveratrol, 1,2-Dimethylhydrazine, Aberrant crypt foci, Chemoprevention, Colon carcinogenesis.

INTRODUCTION

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), a polyphenolic phytoalexin with a relatively broad distribution in plants and in human foods such as skin and seeds of grapes, peanuts and mulberries, has been reported to possess a wide range of biological and pharmacological activities including antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic effects (1). Traditional Japanese and Chinese folk medicines used many herbs for the treatment of liver, skin and circulatory diseases, which are found to be rich in resveratrol (2). Jang and co-workers reported that, resveratrol acts as a pleiotropic biological regulator for each of the three stages-initiations, promotion and progression of malignant transformation using a mouse skin cancer model (3). Recently, resveratrol has also been shown to have cancer chemopreventive activity as it was observed to inhibit *in-vitro* growth of a number of human cancer cell lines, including breast cancer cell lines such as MCF7, MCF10, T47D, MDA-MB-231, ascites hepatoma and Lewis lung carcinoma (4).

1,2-Dimethylhydrazine (DMH) induced aberrant crypt foci (ACF) are readily visible morphological changes within the colonic mucosa that may represent a critical event in the stepwise progression of colon cancer in humans and rats (5). Most of the studies so far have been focused on the beneficial effects of resveratrol in prevention of atherosclerosis, coronary heart disease (6), and little has been considered for its possible use as a cancer chemopreventive agent, especially on a colon carcinogenesis model.

Hence, the aim of our present investigation was to find out if resveratrol supplementation at various stages of carcinogenesis had any effect on ACF development, and subsequently on the incidence of tumors.

MATERIALS AND METHODS

Chemicals

Trans-resveratrol and 1,2-dimethylhydrazine were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade and obtained from Hi-Media Laboratories, Mumbai. Resveratrol (8 mg/kg body weight) was suspended in 0.1% carboxymethylcellulose (CMC) solution and was given orally using an intragastric tube. DMH was dissolved in 1 mM EDTA, the pH adjusted to 6.5 with 1 mM NaOH and used immediately.

Animals and husbandry

Male adult Wistar rats of body weight 120-140 g were acclimatized to control diet for one week. Animals were maintained as per the principles and guidelines of the Ethical Committee of Animal Care of Annamalai University in accordance with the Indian National Law on animal care and use (Reg. No. 160/1999/CPCSEA). The animals were housed four per polypropylene cages

under controlled conditions of 12 h light/12 h dark cycle, with temperature of 24 ± 2°C and relative humidity of 50 ± 10% till the end of the experimental period (30 weeks).

Experimental design

Rats were assorted into 6 experimental groups. Group 1 rats received basal diet along with 0.1% CMC solution/day/kg body weight p.o. throughout the experiment (Control). Group 2 rats received basal diet + 8 mg/kg body weight resveratrol (RES) suspended in 0.1% CMC solution, p.o. everyday for 30 weeks (Control + RES). Group 3 rats were administered 20 mg/kg body weight DMH s.c. once a week for 15 consecutive weeks (DMH). Group 4 animals were administered DMH as in group 3 and fed resveratrol (8 mg/kg body weight) starting 2 weeks before carcinogen treatment and continued till the end of the last DMH injection (DMH + RES-I). Group 5 animals were administered DMH as in group 3 and resveratrol (8 mg/kg body weight) two days after the last injection of the carcinogen and continued till the end of the experiment (DMH + RES-PI). Group 6 animals were administered DMH as in group 3 and resveratrol (8 mg/kg body weight) starting from the day of carcinogen treatment and continued till the end of 30 weeks (DMH + RES-EP). At the end of the experimental period, animals were sacrificed under ketamine hydrochloride (30 mg/kg body weight).

Tumor analysis and preparation for ACF counting

The entire colon was removed, cut longitudinal from cecum to anus, sandwiched between filter papers, fixed in 10% buffered formalin overnight and then stained with 0.2% methylene blue in saline for 2-3 minutes. Specimens were carefully examined topographically at 40 X magnification under a light microscope. The criteria used to identify the aberrant crypts were previously described (7). The ACF were classified as small (1-3); medium (4-6); and large (>6) by the number of crypts per foci. The total number of ACF/rat was calculated as the sum of the small, medium, and large ACF. The aberrant crypts (AC) multiplicity and their distribution pattern along the colon were scored. To obtain additional information about the morphology, sections containing ACF were marked and embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Statistical analysis

Values are expressed as means ± SD of 10 animals in each group. Data within and between the groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS version 11.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows the tumor incidence, ACF incidence, total number of ACF, number of aberrant crypts (AC), AC/ACF (crypt multiplicity), and percentage of ACF inhibition in experimental groups. Administration of resveratrol for 30 weeks (entire period) significantly decreased (25%) the percentage of tumor incidence as compared to DMH-alone treated rats (87%). Similarly, resveratrol supplementation for the entire period of the study caused significant decrease in the total number of ACF, AC/ACF and percentage of inhibition. The majority of the ACF appeared in the middle and distal colon of all carcinogen treated rats (groups 3-6). Distribution of ACF (proximal, middle and distal colon) and AC category (small, medium and large crypts) were significantly reduced in all groups supplemented with resveratrol, especially, the strongest effects were noted in group 6 rats (Table 2).

DISCUSSION

Colon carcinoma is a common cause of death by cancer (70 - 90%) (8). Experimental animal models of colon cancer using azoxymethane and dimethylhydrazine are widely used to investigate the effects of various dietary components on colon carcinogenesis (9).

In the present study, resveratrol exerted a strong inhibitory effect on tumor growth in rat colon carcinogenesis induced by DMH. The ability of resveratrol to reduce cell proliferation (10) and to induce apoptosis in tumors (11) may be the possible mechanism involved. Total number of ACF, number of large ACF, and number of AC/ACF (crypt multiplicity) were used to evaluate the potential colon cancer preventive agents (12). Accordingly, we have also studied ACF in detail and our results suggest that the resveratrol not only inhibited the growth of ACF by decreasing the total number of ACF consisting of various numbers of crypts (small, medium and large), but also inhibited the ACF distribution in proximal, middle and distal regions of colon. Although the mechanisms involved in the protective effects of resveratrol against tumor and ACF formation are not clearly understood, the inhibitory action of resveratrol could be explained as follows, resveratrol is known to (i) affect bax and $P21^{CIP}$ expression in both ACF and surrounding mucosa (13); (ii) inhibit ribonucleotide reductase (14), inhibit DNA polymerase activity (15), inhibit protein kinase C and cyclooxygense-2 activities (16, 17); (iii) inhibit reactive oxygen species (ROS)-

Table 1.- Effect of resveratrol and DMH on tumor incidence and ACF formation in rat colon

Treatment groups	Tumor incidence (%)	ACF formation in rat colon				
		ACF incidence (%)	Total no. of ACF	No. of AC	Crypt multiplicity (AC/ACF)	% of ACF inhibition
DMH	87	10/10 (100)	100.3 ± 10.2^{a}	180.5 ± 14.5^{a}	1.8 ± 0.08^{a}	-
DMH + RES (I)	56	10/10 (100)	50.4 ± 5.3^{b}	85.6 ± 7.6^{b}	1.7 ± 0.08^{ab}	49.75
DMH + RES (PI)	44	10/10 (100)	39.4 ± 5.4^{c}	63.0 ± 20.9^{c}	1.6 ± 0.08^{b}	60.71
DMH + RES (EP)	25	10/10 (100)	28.5 ± 5.1^{d}	37.0 ± 14.3^{d}	1.3 ± 0.07^{c}	71.58

RES, resveratrol; I, initiation; PI, post-initiation; EP, entire period.Data is presented as means ±SD of 10 rats in each group. Groups not sharing a common superscript letter (a-d) differ significantly at P < 0.01 (DMRT).

Table 2. - Effect of resveratrol and DMH on ACF distribution in proximal, middle and distal rat colon

ACF distribution	DMH	DMH + RES (I)	DMH + RES (PI)	DMH + RES (EP)
Proximal colon	11.7 ± 0.9^{a}	6.7 ± 0.6^{b}	4.2 ± 1.3^{c}	1.7 ± 0.6^{d}
Small	9.2 ± 0.6	4.1 ± 0.3	3.2 ± 1.0	1.0 ± 0.4
Medium	1.5 ± 0.2	1.4 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Large	1.0 ± 0.1	1.2 ± 0.1	0.3 ± 0.1	-
Middle colon	30.1 ± 3.2^{a}	14.3 ± 1.6^{b}	12.3 ± 1.5^{c}	10.2 ± 1.6^{d}
Small	11.1 ± 1.2	8.3 ± 1.0	8.2 ± 1.0	2.8 ± 0.4
Medium	15.3 ± 1.9	5.0 ± 0.4	2.6 ± 0.3	5.3 ± 0.9
Large	3.7 ± 0.1	1.0 ± 0.2	1.5 ± 0.2	2.1 ± 0.3
Distal colon	58.5 ± 6.1^{a}	29.4 ± 3.1^{a}	22.9 ± 2.6^{bc}	16.6 ± 2.9^{c}
Small	15.3 ± 1.6	13.3 ± 1.9	6.2 ± 0.3	8.3 ± 1.5
Medium	25.6 ± 3.5	10.1 ± 0.5	10.8 ± 1.6	4.2 ± 0.5
Large	17.6 ± 1.0	6.0 ± 0.7	5.9 ± 0.7	4.1 ± 0.9

RES, resveratrol; I, initiation; PI, post-initiation; EP, entire period. Data is presented as means ±SD of 10 rats in each group. Groups not sharing a common superscript letter (a-d) differ significantly at P < 0.01 (DMRT).

mediated carcinogenesis (3); (iv) inhibit tumor cell division (18); and (v) activate apoptotic cell death (11).

Thus conclusively, our results provide evidence that continuous exposure to dietary resveratrol attenuates tumor development/incidence and ACF formation in DMH-induced rat colon carcinogenesis. Moreover, resveratrol supplementation during the entire period of the study was more effective as compared to resveratrol supplementation during the initiation or post initiation stages of colon carcinogenesis.

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