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Inhibitory effect of peptide Epitalon on colon carcinogenesis induced by 1,2-dimethylhydrazine in rats

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Abstract

The effect of synthetic pineal peptide Epitalon (Ala-Glu-Asp-Gly) on colon carcinogenesis was firstly studied in rats. Eighty 2-month-old outbred male LIO rats were subdivided into four groups and were weekly exposed to five subcutaneous injections of 1,2-dimethylhydrazine (DMH) at a single dose of 21 mg/kg body weight. Additionally, 5 days a week, some of the rats were given subcutaneous injections of saline at a dose of 0.1 ml during the whole experiment (group 1, control) or Epitalon at a single dose of 1 μg during the whole experiment (group 2), Epitalon after termination of carcinogen injections (group 3) or during the period of DMH exposure (group 4). Colon carcinomas developed in 90–100% of DMH-treated rats. The number of total colon tumors per rat was 4.1; 2.7; 3.7; 2.9 in groups 1, 2, 3, 4, respectively (the difference in groups 2 and 4 compared with group 1 is significant). In rats from group 2, colon tumors were smaller than in control animals. In group 2, the incidence, as well the multiplicity of tumors in ascending and descending colon, were significantly decreased in comparison with group 1. In group 4, the mean number of tumors per rat was significantly decreased, too. A trend to decrease the number of tumors in the rectum in rats from groups 2, 3 and 4, treated with Epitalon was found. Epitalon inhibited also the development of tumors in jejunum and ileum. Thus, our results demonstrated an inhibitory effect of Epitalon on chemically induced bowel carcinogenesis in rats.

Keywords: Colon carcinogenesis; 1,2-Dimethylhydrazine; Peptide; Epitalon

1. Introduction

In the last decades, data have accumulated concerning the role of the pineal gland in tumor development [1,2]. In cancer patients, a decrease in pineal function was found. Pinealectomy stimulated tumor growth in animals. At the same time, pineal hormone melatonin and the pineal peptide preparation Epithalamin inhibited the development of spontaneous and chemically induced neoplasms [2–6]. Recently, the synthetic pineal tetrapeptide Epitalon (Ala-Glu-Asp-Gly) has been structured on the basis of the amino acid analysis of the pineal preparation Epithalamin, and was tested in CBA mice [7]. It was found that this compound reduced the incidence of spontaneous tumors [7].

To evaluate the anticarcinogenic potential of Epitalon, it is necessary to test this compound in different tumor models. Among these models, colon cancer

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seems to be one of the most significant. This cancer is widely distributed around the world [8]. In colon cancer patients the disturbances in pineal function have been observed [1,9]. There are experimental data on the relationship between the pineal gland and cell proliferation in the gastrointestinal tract [3,10]. An effect of Epitalon on the cell metabolism in intestinal mucosa has been shown [11]. Melatonin inhibited colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) in rats [3,5].

In this paper, the results of a study on the effect of Epitalon on DMH-induced colon carcinogenesis in rats are presented.

2. Materials and methods

2.1. Chemicals

1,2-Dimethylhydrazine dihydrochloride (DMH) was from Sigma (USA) and was kept at −20 °C. Epitalon was synthesized by G.I. Grigoriev, St. Petersburg Institute of Bioregulation and Gerontology, Russia.

2.2. Animals

Two-month-old outbred male LIO rats from the Animal Department of Central Research Roentgeno-Radiologocal Institute, St. Petersburg, were used in the study. The characteristics of these rats have been described elsewhere [12]. Animals were kept five per polypropylene cage under a standard light/dark regimen (12:12 h light/darkness) at 21–23 °C. They received standard laboratory chow [13] and tap water ad libitum.

2.3. Experiment

Eighty animals were randomly subdivided into four groups. All rats were exposed weekly to five subcutaneous injections of DMH at a single dose of 21 mg/kg of body weight (calculated as a base). In this regimen, the carcinogen induced colon tumors in the majority of rats [3,5]. DMH was ex tempore dissolved in normal saline and neutralized with sodium bicarbonate (pH 7.0). Additionally, 5 days a week, rats from group 1 (control) were injected with 0.1 ml of normal saline, and rats from groups 2, 3 and 4 with 1 μg of Epitalon dissolved in 0.1 ml of saline. Animals from groups 1 and 2 received saline and Epitalon, respectively, during the whole experiment. Rats from group 3 were exposed to Epitalon after termination of DMH injections, and rats from group 4 during the period of DMH exposure. For standardization of stressful procedures associated with injections, rats from group 3 (during the period of DMH exposure), and group 4 (after termination of DMH injections) received the saline subcutaneously, 5 days a week. The design of the experiment is given in Fig. 1. All animals were weighed weekly. The experiment was finalized 6 months after the first injection of the carcinogen, and all rats were killed by ether vapor.

2.4. Pathological investigation

All animals were autopsied. Intestines were opened longitudinally. The position and size of each tumor were recorded on special charts [14]. All tumors and other tissues with macroscopically revealed lesions were fixed in 10% neutral formalin and, after routine histological processing, were embedded in paraffin. Five-to-seven-micrometer-thick sections through the middle part of each tumor were stained with hematoxylin and eosin. The neoplasms were classified according to the IARC recommendations [15].

2.5. Statistics

Experimental results were statistically processed according to IARC recommendations [16]. Tumor incidence was evaluated for significance by Fisher's exact method [17]; differences in animal body weight, tumor size and multiplicity were determined by Wilcoxon's *U*-test and Student's *t*-test [17].

3. Results

DMH and Epitalon treatment does not influence animal status. There were no significant differences in the body weight between different groups. At the end of experiment, intestinal tumors were found in the majority of rats (Table 1). Macroscopically, these neoplasms were exophytic or endophytic. Several cases of ulcerative-infiltrative forms were observed as well. Microscopically, different types of malignant intestinal tumors were found, predominantly, tubular

adenocarcinomas. In situ, superficial carcinomas, mucinous and signet-ring carcinomas were also registered. All these types of carcinoma are typical for neoplasms induced by DMH [14,18]. The majority of carcinomas (61–78% in different groups, the largest incidence in group 2) were highly differentiated tumors. Invasion confined to lamina propria of the mucosa was observed in 5–14% of tumors in different groups, invasion into the submucosa in 40–65% of neoplasms, invasion into the muscular coat in 18–25%, and into the serosa in 10–23%. Metastases to the mesentery and greater omentum appeared in one rat from group 1, one animal from group 3 and two rats from group 4. The differences between groups

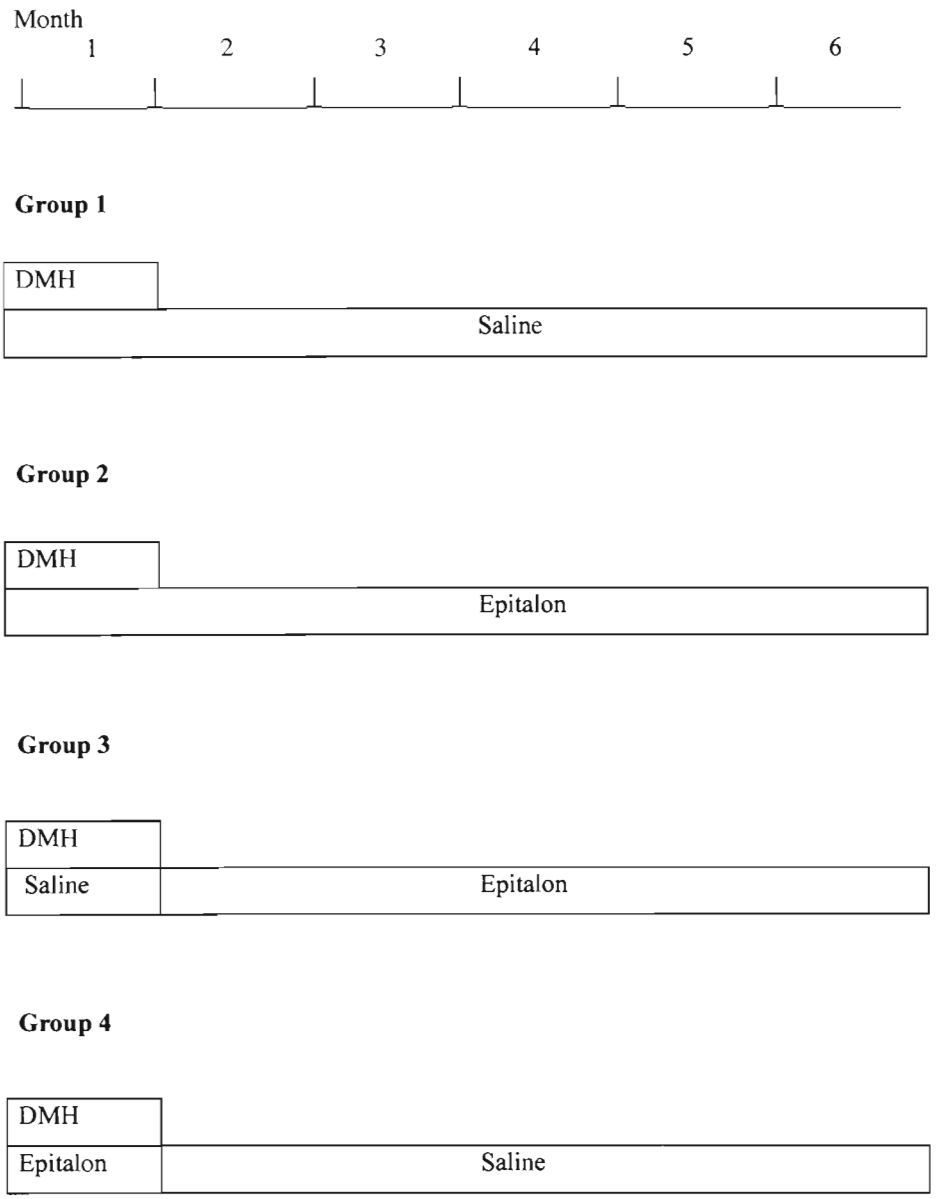

Fig. 1. The design of the experiment.

were not significant; however, some trend towards an increase in differentiation level was observed among carcinomas induced in rats from group 2.

The majority of intestinal malignancies were tumors of the large intestine (Table 1). Total incidence of these tumors in different groups was quite similar (90–100%). However, the multiplicity (mean number of tumors per rat in a group) in animals from groups 2 and 4 that received DMH + Epitalon was significantly lower than in rats exposed to DMH alone. The mean number of tumors per tumor-bearing rat was also decreased in rats from group 2 in comparison with the control one.

The results of cluster analysis of distribution of animals with different numbers of colon tumors are presented in Table 2. In control animals (group 1) only multiple tumors (three and more) developed, whereas in rats treated by Epitalon (groups 2 and 3) single carcinomas were frequently observed. The highest incidence of single tumors was observed in group 2.

Table 1
Effect of Epitalon on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in rats[a]

Parameter	Group 1: DMH	Group 2: DMH + Epitalon (whole experiment)	Group 3: DMH + Epitalon (after DMH)	Group 4: DMH + Epitalon (during DMH exposure)
No. of rats	20	20	20	20
No. of tumor-bearing rats	20 (100%)	19 (95%)	19 (95%)	18 (90%)
Localization of tumors				
Total colon				
No. of tumor-bearing rats	20 (100%)	19 (95%)	19 (95%)	18 (90%)
No. of tumors	81	53	74	58
No. of tumors per rat:				
per rat in group	4.1 ± 0.42	2.7 ± 0.42*	3.7 ± 0.54	2.9 ± 0.36*
per tumor-bearing rat	4.1 ± 0.42	2.8 ± 0.38*	3.9 ± 0.50	3.2 ± 0.32
Mean tumor size, mm^2	56.9 ± 4.7	35.8 ± 3.6*	48.8 ± 7.6	56.5 ± 13.9
Ascending colon				
No. of tumor-bearing rats	17 (85%)	12 (60%)**	14 (70%)	15 (75%)
No. of tumors	29	19	32	25
No. of tumors per rat:				
per rat in group	1.5 ± 0.18	1.0 ± 0.18*	1.6 ± 0.30	1.3 ± 0.18
per tumor-bearing rat	1.7 ± 0.14	0.6 ± 0.18**	2.3 ± 0.31***	1.7 ± 0.15***
Mean tumor size, mm^2	56.9 ± 8.4	54.0 ± 29.2	63.3 ± 12.8	50.2 ± 15.0
Descending colon				
No. of tumor-bearing rats	20 (100%)	14 (70%)**	16 (80%)	15 (75%)
No. of tumors	45	35	39	33
No. of tumors per rat:				
per rat in group	2.3 ± 0.24	1.8 ± 0.30**	2.0 ± 0.30	1.7 ± 0.18*
per tumor-bearing rat	2.3 ± 0.24	2.5 ± 0.31	2.4 ± 0.28	2.2 ± 0.15
Mean tumor size, mm^2	51.0 ± 7.0	33.8 ± 5.0**	41.1 ± 8.3	61.3 ± 20.2
Rectum				
No. of tumor-bearing rats	4 (20%)	2 (10%)	1 (5%)	0
No. of tumors	7	2	2	–
No. of tumors per rat:				
per rat in group	0.4 ± 0.18	0.1 ± 0.06	0.1 ± 0.06	–
per tumor-bearing rat	1.8 ± 0.49	1.0	2.0	–
Mean tumor size, mm^2	27.5 ± 16.2	26.8 ± 23.6	45.5 ± 21.6	–

[a] The difference with group 1 is significant: *$P < 0.05$, **$P < 0.01$; the difference with group 2 is significant: ***$P < 0.05$.

Table 2
Distribution of rats with different number of colon tumors in the experiment with DMH and Epitalon[a]

Parameter	Group 1: DMH	Group 2: DMH + Epitalon (whole experiment)	Group 3: DMH + Epitalon (after DMH)	Group 4: DMH + Epitalon (during DMH exposure)
No. of rats	20	20	20	20
No. of rats with colon tumors	20 (100%)	19 (95%)	19 (95%)	18 (90%)
No. of rats with (% no. of rats in group):				
1 tumor	–	6 (30%)*	3 (15%)	2 (10%)
2 tumors	–	3 (15%)	3 (15%)	4 (20%)
3–5 tumors	15 (75%)	9 (45%)*	8 (40%)**	10 (50%)
6 and more tumors	5 (25%)	1 (5%)*	5 (25%)	2 (10%)

[a] The difference with group 1 is significant: $^{**}P < 0.05$, $^{**}P < 0.03$.

Among carcinomas in different parts of the large intestine, tumors of descending colon prevailed (Table 1). Its incidence in group 2 was significantly decreased in comparison with group 1. Such a trend was also observed in groups 3 and 4. The tumor multiplicity in rats from groups 2 and 4 was significantly lower in comparison with animals from group 1.

Tumors of the ascending colon appeared in the control group more often than in animals exposed to Epitalon. In rats from group 2 this difference was significant. Among these rats, the tumor multiplicity decreased compared with group 1. Tumors of the rectum developed in groups 1–3 only. Its incidence in rats from groups 2 and 3 was somewhat lower than in the control group.

Mean size of total colon tumors was significantly decreased in rats from group 2, mainly due to differences in size of tumors of the ascending colon (Table 1). Analysis of tumor size distribution (Table 3) has shown that in the ascending colon of animals from group 3 small tumors (11–50 mm^2) appeared less frequently in comparison with the control group, whereas in group 4 large tumors (51–100 mm^2) developed more frequently than in group 1. It should be mentioned that there were only four large tumors of ascending colon in group 4. In descending colon, tumor size distribution in different groups was similar.

Malignancies of the small intestine were much rarer than tumors of the large intestine (Table 4). It was found that the incidence of tumors in the jejunum and in the ileum among rats treated with Epitalon was low in comparison with controls, and in animals from group 4 this difference was significant.

Concerning pathology in other organs, the main lesions were found in the liver. In some animals, bile duct and oval cell proliferation was observed. In two rats from group 1, two from group 3 and two from group 4, cystic cholangiomas appeared. Single cases of lymphoid hyperplasia, as well as of pneumonia, were also found

4. Discussion

The aim of present study was to evaluate the effect of the synthetic pineal peptide Epitalon on colon cancer development. In the study we used one of the most popular experimental models of cancer of the large intestine: carcinogenesis induced by DMH in rats. It was shown that DMH-induced colon carcinomas are morphologically similar to human colon carcinomas, and like other malignancies, a colon carcinoma needs at least several stages (events) for development [14,18]. In this model, earlier we studied the effect of pineal hormone melatonin on colon cancer development [3,5]. To evaluate the effect of Epitalon on different stages of carcinogenesis, in the present study the different regimens of Epitalon treatment were used: during initiation stage (group 4), stage of tumor promotion (group 3), or the whole process of tumor development (group 2).

Our experiments demonstrated the inhibitory effect of Epitalon on colon cancer development at various stages of carcinogenesis. This effect was manifested mainly by a decrease in the incidence and multiplicity of bowel tumors. In animals from group 2 the mean size of colon tumors was decreased. The inhibitory effect of Epitalon on bowel carcinogenesis was

Table 3
Size distribution of colon tumors in the experiment with DMH and Epitalon[a]

Tumor size (mm^2)	Group 1: DMH	Group 2: DMH + Epitalon (whole experiment)	Group 3: DMH + Epitalon (after DMH)	Group 4: DMH + Epitalon (during DMH exposure)
Ascending colon				
≤ 10	2 (6.9%)	4 (22.2%)	3 (9.0%)	5 (20.0%)[b]
11–50	16 (55.2%)[a]	7 (38.9%)	11 (33.3%)*[a]	13 (52.0%)
51–100	6 (20.7%)[b]	6 (33.3%)	15 (45.5%)*	4 (16.0%)[b]
> 100	5 (17.2%)[b]	1 (5.6%) [b]	4 (12.1%)[c]	3 (12.0%)[b]
Descending colon				
≤ 10	10 (22.2%)	9 (28.1%)	11 (28.2%)	10 (30.0%)
11–50	23 (51.1%)[b]	14 (43.8%)	17 (43.6%)	14 (42.4%)
51–100	5 (11.1%)[b]	8 (25.0%)	8 (20.5%)	6 (18.2%)[b]
> 100	7 (15.6%)[b]	1 (3.1%)[b]	3 (7.7%) [b]	3 (9.1%)[ab]
Rectum				
≤ 10	3 (42.9%)	1 (50.0%)	–	–
11–50	3 (42.9%)	1 (50.0%)	1 (50.0%)	–
51–100	–	–	1 (50.0%)	–
> 100	1 (14.3%)	–	–	–

[a] The difference with group 1 is significant: $^*P < 0.05$; the difference with parameter for tumors ≤ 10 mm^2 inside the group is significant: $^aP < 0.05$; the difference with parameter for tumors 11–50 mm^2 inside the group is significant: $^bP < 0.05$; the difference with parameter for tumors 51–100 mm^2 inside the group is significant: $^cP < 0.05$.

Table 4
Effect of Epitalon on DMH-induced small intestine carcinogenesis in rats[a]

Parameter	Group 1: DMH	Group 2: DMH + Epitalon (whole experiment)	Group 3: DMH + Epitalon (after DMH)	Group 4: DMH + Epitalon (during DMH exposure)
No. of rats	20	20	20	20
Localization of tumors				
Duodenum				
No. of tumor-bearing rats	2 (10%)	1 (5%)	4 (20%)	2 (10%)
No. of tumors	2	1	5	2
No. of tumors per rat:				
per rat in group	0.10 ± 0.06	0.05 ± 0.06	0.25 ± 0.12	0.10 ± 0.06
per tumor-bearing rat	1.00	1.0	1.25 ± 0.08	1.0
Mean tumor size, mm^2	65.9 ± 35.3	62.8	76.8 ± 16.9	79.3 ± 2 8.5
Jejunum and ileum				
No. of tumor-bearing rats	7 (35%)	3 (15%)	3 (15%)	1 (5%)*
No. of tumors	7	3	3	2
No. of tumors per rat:				
per rat in group	0.35 ± 0.06	0.15 ± 0.06	0.15 ± 0.06	0.10 ± 0.06
per tumor-bearing rat	1.0	1.0	1.0	2.0
Mean tumor size, mm^2	108.0 ± 24.7	115.4 ± 50.8	77.5 ± 38.7	127.6 ± 61.33

[a] The difference with group 1 is significant: $^*P < 0.01$.

comparable with the effect of melatonin [3,5]. Our data confirmed the hypothesis on the preventive role of the pineal gland in cancer development, realized through the action of melatonin as well as of the pineal peptide(s).

A comparison of the anticarcinogenic effect of Epitalon in different animal groups has shown that it is most effective on exposure to the preparation during the whole experiment, i.e. both initiation and promotion stage of carcinogenesis (group 2). It is well known that free radicals play a significant role in tumor initiation and promotion [19,20]. Antioxidants inhibit carcinogenesis induced by DMH [21]. It should be mentioned that both the pineal peptide preparations Epithalamin and Epitalon have antioxidant properties [7,22]. Thus, the anticarcinogenic effect of the pineal peptide Epitalon could be explained by the possible antioxidant properties of this compound. It was found that Epitalon inhibits cell proliferation [23], and this is a well-known component of the anticarcinogenic effect. It is supposed that Epitalon can act both directly and indirectly through the stimulation of pineal function [7,24].

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