

Effect of the Synthetic Pineal Peptide Epitalon on Spontaneous Carcinogenesis in Female C3H/He Mice

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Abstract. *The potential preventive effect of the synthetic pineal peptide Epitalon[®] (Ala-Glu-Asp-Gly) on spontaneous tumorigenesis in mice was studied. One-year-old female C3H/He mice were kept for 6.5 months under standard conditions. Epitalon was injected at a dose of 0.1 µg, 5 times a week. Long-term exposure to Epitalon in small doses did not show any toxic effect. Treatment with Epitalon decreased the number of tumor-bearing mice with malignant tumors and prevented the development of metastases. Spontaneous tumors of the reproductive organs (mammary glands and ovaries) were predominant in both groups of mice (control and experimental). The mammary gland tumors were different variants of invasive ductal carcinomas. In the ovaries, granulosa-cell tumors were found. Tumors were in the minority in other organs and had benign characteristics. In control mice, metastases were found in 3 out of 9 tumor-bearing mice, all of them being from tumors of the reproductive organs. Treatment with Epitalon slowed down the development of metastases from spontaneous tumors, and no metastases were found in the experimental mice. These data highlight the antimetastatic effect of Epitalon as part of its oncostatic properties.*

Pineal hormones affect the activity of the female reproductive organs and play a significant role in the general protection against different foreign factors, including carcinogens. The pineal hormone melatonin and the pineal peptide preparation Epithalamin have been

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shown to inhibit the development of spontaneous and chemically-induced neoplasms (1,2), especially of hormone-dependent mammary tumors (3-5). Epitalon[®], the synthetic derivative of Epithalamin, has also been found to reduce the incidence of spontaneous tumors in inbred CBA and HER-2/neu transgenic mice, as well as in Swiss-derived SHR mice, and of chemically-induced colon tumors in rats (6-10).

The effect of the synthetic peptide Epitalon on spontaneous carcinogenesis in inbred C3H/He mice was studied. The findings add to our understanding of the role of pineal hormones in the pathogenesis of cancer.

Materials and Methods

Chemicals. The tetrapeptide Epitalon (Ala-Glu-Asp-Gly) was synthesized on the basis of the amino acid sequence of Epithalamin by G.I. Grigoriev, St. Petersburg Institute of Bioregulation and Gerontology, Russia.

Animals. Twice-bearded one-year-old female C3H/He mice (n=117, Harlan Labs., Jerusalem, Israel) were used in the experiment. The animals were kept, 5 per polypropylene cage, under a standard 12-h light/12-h darkness regimen at 21° to 23°C. Standard laboratory chow and tap water were available *ad libitum*.

Tumorigenic experiment. The animals were divided into 2 groups: control (n=56) and experimental (n=61). Mice in the control group received sterile saline at a dose of 0.2 ml/mouse, subcutaneously (*s.c.*) 5 days a week. Mice in the experimental group received Epitalon *s.c.* on the same days at a dose of 0.1 µg dissolved in 0.2 ml saline/mouse. The experiment was terminated 6.5 months after the first injection of Epitalon, and the mice were killed by cervical dislocation.

Scoring and analysis. All the animals were checked visually 5 days a week and weighed monthly throughout the experimental period. They were autopsied after 6.5 months. The position and size of each tumor were recorded. All tumors were fixed in 4% neutral formaldehyde and, after routine histological processing, were embedded in paraffin. Sections (3-µm-thick) through the

middle part of each tumor were stained with hematoxylin and eosin (H&E). All data were analyzed statistically by the Student's *t*-test.

Results

Long-term exposure to Epitalon in small doses did not show any toxic effects, and no mortalities were recorded. During the experiment, no changes were found in the weight of the experimental mice *versus* that of their control counterparts.

Although the experiment (6.5 months) was not long enough to determine the effect of Epitalon on spontaneous tumorigenesis, its results are expected to be of interest to experimental oncologists.

Treatment with Epitalon decreased the number of tumor-bearing mice with malignant tumors and prevented the development of metastases (Table I). Spontaneous tumors of the reproductive organs (mammary glands and ovaries) were predominant in both groups of mice (control and experimental) (Figures 1A, 2A). Mammary-gland tumors presented different variants of invasive ductal carcinomas, while ovarian tumors were of the granulosa-cell type. Tumors in the other organs were in the minority and had benign characteristics: trichofolliculomas and trichoepitheliomas in the skin, and adenomas in the lungs and liver.

In control mice, metastases were found in 3 out of 9 tumor-bearing mice, all of them being from tumors of the reproductive organs (Table I). In 2 mice, metastases of mammary tumors were found in the lungs (Figure 1A,B), and in 1 mice, a metastasis of an ovarian tumor was found in the neighboring fat tissues (Figure 2A,B). No metastasized tumors were found in the experimental mice.

Discussion

The aim of the present study was to evaluate the effect of the synthetic pineal peptide Epitalon on spontaneous tumorigenesis in female C3H/He mice. Spontaneous mammary tumors and their metastases in C3H mice have been described previously (11). Among spontaneous ovarian tumors in these mice, granulosa-cell tumors predominate (12). In transgenic FVB/N mice carrying the breast cancer gene HER-2/neu, Epitalon prolonged the average lifetime of animals without neoplasms by 34.2%, decelerated the development of age-related disturbances in reproductive activity and suppressed the formation of neoplasms (7). The peptide decreased the incidence of breast adenocarcinomas, lung metastases and multiple tumors, increased the number of mice without breast tumors, and prolonged the lifetime of mice with breast tumors by 1.4-fold, compared to their control counterparts. In outbred Swiss-derived SHR mice, Epitalon increased the

Table I. Incidence, localization and type of tumors in mice treated and not treated with Epitalon.

Parameters studied	Saline	Epitalon
Total number of mice	56	61
Number of tumor-bearing mice	9 (16.1%)	7 (11.5%)
Number of malignant tumor-bearing mice	6 (10.7%)	3 (4.9%)
Total number of tumors	9	7
Number of metastasizing tumors	3	-
Localization and type of tumors:		
Mammary gland: invasive ductal carcinoma	5 (2) ^a	3
Ovaries: granulosa-cell tumor	1 (1) ^a	1
Skin: trichofolliculoma	2	-
trichoepithelioma	-	2
Lungs: adenoma	1	-
Liver: hepatoma	-	1

^aIn parentheses, the number of mice with metastases.

maximum lifespan by 12.3% in comparison with the control group (8). Although Epitalon treatment did not influence the total incidence of spontaneous tumors, it inhibited the development of leukemia by 6-fold relative to the control group. In tumor-bearing LIO rats exposed to 1,2-dimethylhydrazine, Epitalon at a daily dose of 1 µg/rat throughout the course of the experiment significantly decreased the number of tumors in the colon compared to the controls, and inhibited the development of tumors in the jejunum and ileum (9). Moreover, Epitalon inhibited the mitotic activity of both the epithelial cells adjacent to tumors and the tumor cells themselves when the treatment was given throughout the experiment (10). In parallel, a high level of apoptosis was seen in this group of rats. Melatonin or Epitalon treatment was followed by longer mean and maximum survivals in 10% of the last survivors among senescence-accelerated mice (SAM) (13). It should be noted that the anticarcinogenic effect of Epitalon was most effective when it was administered throughout the experiment, *i.e.*, at both the initiation and promotion stages of carcinogenesis (6-8).

The antitumorigenic effects of Epitalon and melatonin are manifested in their inhibition of cell proliferation (4,14). Among the different mechanisms that have been described to date governing the effects of these pineal components (3), their anti-invasive and antimetastatic activities have been least studied, despite the opinion that these parameters are an important part of the oncostatic action of these components (15). It has been shown, for example, that melatonin as a supplementary treatment to chemotherapy promotes the 5-year survival rate of non-small cell lung cancer patients with metastases (16).

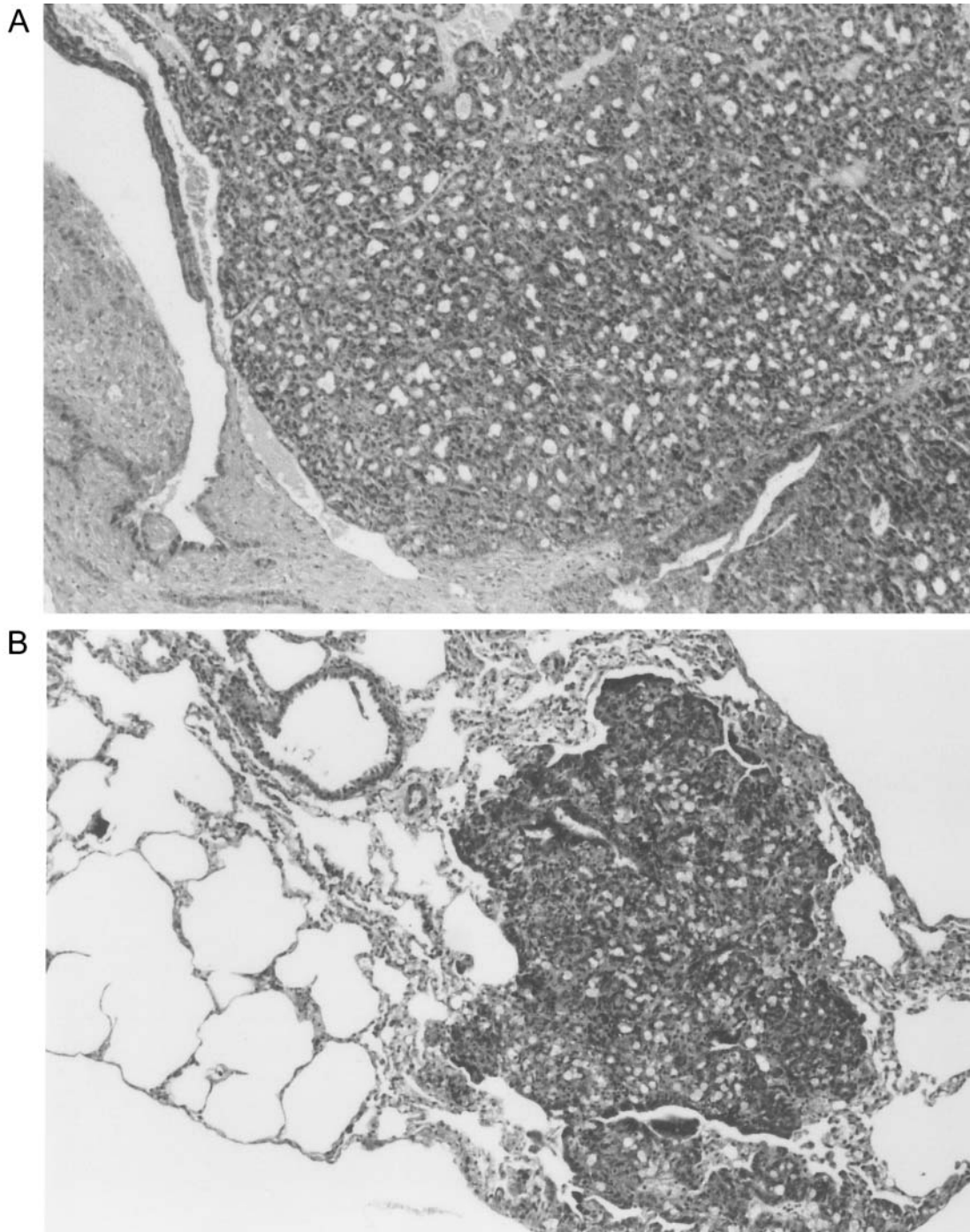


Figure 1. Tumor of the mammary gland. H&E. x80. A, Invasive ductal carcinoma. B, Metastasized tumor in the lungs.

This study showed that Epitalon decreased the number of tumor-bearing mice with malignant tumors and prevented the metastasis of spontaneous tumors from the mouse reproductive organs. The antitumor effect of Epitalon appeared to be similar to those of its natural analogs (3).

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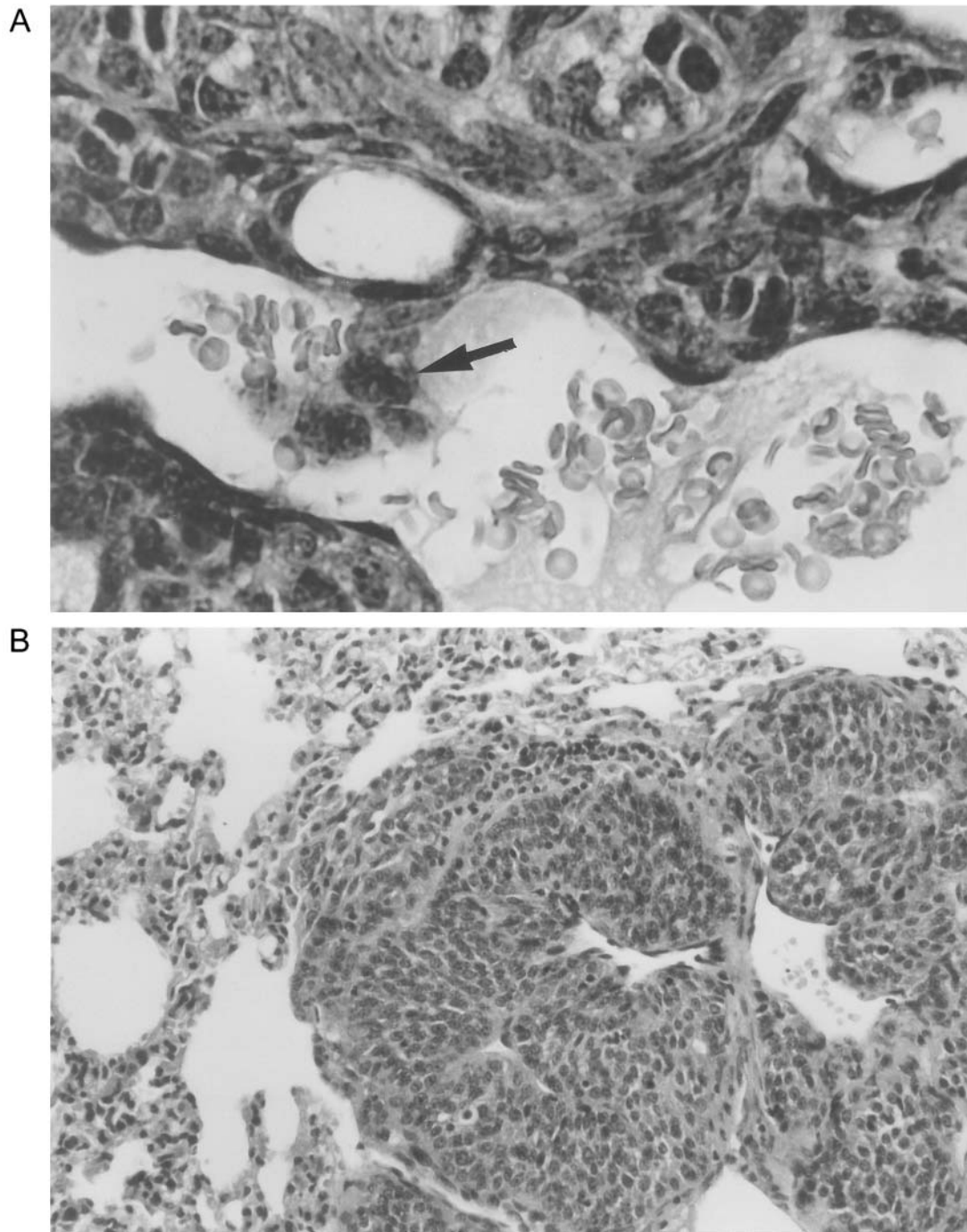


Figure 2. Tumor of the ovary. H&E. A, x800. B, x200. A, Granulosa-cell tumor. Note invasive lodgement of tumor cells in a blood vessel (arrow). B, Metastasized tumor in the lungs.

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