

Study of the Anti-Tumor Effect of Polypeptide Pineal Extract

V.M. Dilman, V.N. Anisimov, M.N. Ostroumova, V.G. Morosov, V.Kh. Khavinson and M.A. Azarova

Laboratory of Endocrinology, Petrov Research Institute of Oncology, Leningrad

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Abstract. Bovine pineal polypeptide extract (PPE) exerted an anti-tumor effect on mouse-transplantable tumors: mammary cancer (RSM), squamous cell cervical carcinoma (SCC), hepatoma-22a and lympholeukemia LIO-1, and had no effect on Harding-Passey melanoma and leukemia L-1210. It was shown that PPE possessed the ability to decrease the incidence of DMBA-induced mammary adenocarcinomas in rats. The daily administration of 0.5 mg PPE prolonged the life span of rats by 25% and failed to influence spontaneous tumor development. The arguments in favor of a possible mechanism of anti-tumor action of the pineal gland are submitted. It is suggested that the anti-tumor effect of PPE may occur when the syndrome of cancrophilia is induced by tumor transplantation or chemical carcinogens.

Introduction

It should be stressed that the elevation of the threshold of hypothalamic sensitivity to feedback control may be interpreted as a key mechanism of body development, aging and age-connected pathology [16, 17]. The phenomenon of hypothalamic threshold elevation is also observed in stress, mental depression and some forms of cancer [16, 18, 19, 37, 49], as well as after treatment with many types of chemical carcinogens [5]. There is every reason to believe that this phenomenon develops as a result of a number of factors, e.g., the decline in biogenic amine levels in the hypothalamus, the decrease of binding of hormones to their specific receptors, the decline in pineal gland function [8, 17, 24, 28].

Our previous experiments have shown that polypeptide pineal extract (PPE) increases the hypothalamus-pituitary sensitivity to the inhibiting effect of prednisolone and stilbestrol [3, 6, 39]. Undoubtedly, these extracts cause a number of other effects, which at present cannot be completely recognized. In general, the pineal gland exerts a suppressive action on some functions of the hypothalamus-pituitary system as well

as on a number of other systems [13, 28, 43]. On the other hand, there is evidence pointing to the influence of the pineal gland on the fat-carbohydrate metabolism [34]. In particular, it was shown in our laboratory that PPE raises carbohydrate tolerance and reduces blood insulin and the triglyceride level [41]. The effects of PPE on the threshold of sensitivity of the hypothalamus-pituitary complex to feedback control and metabolic pattern seem to be interrelated. To specify, the lipid-carbohydrate metabolism influences serotonin levels in the brain and hypothalamus [23] which, in turn, may affect the sensitivity of this system to regulatory stimuli. Considering these newly revealed aspects of the action of pineal polypeptide hormones, we decided to reconsider some aspects of the problem of interrelationships of the pineal gland and carcinogenesis.

There is a great body of evidence that after pinealectomy the growth and metastatic spread of transplantable tumors are intensified [15, 31, 44]. At the same time, administration of melatonin [4, 12, 21] or pineal extracts [4, 11, 22, 29, 32, 35, 40] suppresses tumor development or tumor growth in animals. Clinical studies showed histological changes of the pineal

structure in patients who died from cancer [45, 47]. In some endocrine-dependent malignant tumors in man, the excretion of the antigonadotropic factor is decreased; meanwhile, there is some evidence that this factor is produced in the pineal gland [38, 40].

The paper presents data on the effect of polypeptide bovine pineal extract on the (1) growth of a number of transplantable tumors, (2) development of DMBA-induced neoplasms, (3) frequency of spontaneous tumor in rats, and (4) the life span of rats.

Material and Methods

Preparation of PPE

Acetone powder of the bovine pineal glands was extracted during 72 h with 0.1 N acetic acid in the presence of $ZnCl_2$; after centrifugation the supernatant was precipitated by 4–8 vol acetone. The precipitate, dried with acetone and ether, was lyophilized and sterilized [35]. The fractionation of PPE with carboxyl cationite 'Biocarb' (USSR) revealed the three fractions, the contents of which in total extract were 74, 16 and 10%, respectively. In the subsequent study it was found that fractions I, II and III, respectively, had (1) molecular weights of 250, 11,000 and 1,200; (2) α -amine nitrogen contents of 1.40×10^{-3} , 0.34×10^{-3} and 4.0×10^{-3} $\mu\text{mol/g}$; (3) isoelectric points 3.0, 5.0 and 10.5. In all experiments the total preparation of PPE has been used.

1st Series of Experiments

The study of the anti-tumor activity of PPE was carried out on the following mice-transplantable tumor strains: hepatoma-22a ($C_3\text{HA}$), SCC (squamous cell cervical carcinoma) (BALB/c) [9], Harding-Passey melanoma (SHR), RSM (microalveolar mammary cancer) ($C_3\text{HA}$) [42], lympholeukemia LIO-1 (SHR) [34] and leukemia L-1210 ($C57\text{BL}/\text{DBA}2$). Only female mice were used. Melanoma, hepatoma, RSM and SCC strains were transplanted subcutaneously; LIO-1 was transplanted intramuscularly, and L-1210 intraperitoneally by routine procedures. PPE was dissolved in normal

saline, and daily subcutaneous injections were performed for 10 days, starting from the 4th day after transplantation of hepatoma, melanoma, SCC or RSM strains. 24 h after the last injection of PPE the animals were killed and the weight of tumors was determined. LIO-1-inoculated mice received the preparation for 14 days starting from the 6th day of transplantation; L-1210-inoculated mice received the preparation for 14 days starting from the 1st day of transplantation. The terms of death of animals were registered.

2nd Series of Experiments

72 female, 3-month-old rats, provided by Rappolovo breeding farms (AMS, USSR), received 6 intravenous injections with oil-aqueous emulsion of 7,12-dimethylbenz(a)-anthracene (DMBA; Fluka, Buchs, Switzerland) with a 1-week interval. A single dose of the DMBA given was 1.5 mg/rat, the total dose being 9 mg/rat. 1 month before starting the carcinogen administration, during administration, and in the course of all experiments, 35 rats received subcutaneous injections of PPE 5 times per week in a dose of 0.5 mg/day in 0.2 ml saline. 37 control rats were treated with 0.2 ml normal saline. All animals were examined weekly. Tumors were examined histologically.

3rd Series of Experiments

To study the action of chronic administration of PPE, 0.1 or 0.5 mg of the preparation was given subcutaneously in 0.2 ml normal saline to female rats without pretreatment 5 times per week for 20 months, starting at ages of 3–4 months. Control rats received 0.2 ml saline. Autopsies were performed on all animals. Tumors were fixed in 10% neutral formalin, and 7- μm paraffin-celloidin sections were stained with hematoxylin and eosin.

Results

(1) Table I shows that PPE exerted a pronounced effect on the mice inoculated with RSM and SCC tumor cell strains, a weak action on hepatoma-22a, and had no effect on Harding-Passey melanoma. In addition, PPE significantly increased the survival time of SHR

Table I. Effect of PPE on the growth of transplantable tumors in mice

Strain of mice	Tumor strain	Daily dose mg	Number of animals	Average weight of tumor, mg	Tumor inhibition %	p
$C_3\text{HA}$	RSM	0	40	987 ± 187	–	–
		1	16	484 ± 77	51	<0.05
		2	28	198 ± 78	80	<0.01
$C_3\text{HA}$	hepatoma-22a	0	36	604 ± 79	–	–
		2	34	393 ± 73	35	<0.05
BALB/c	SCC	0	34	823 ± 105	–	–
		1	9	610 ± 194	26	>0.05
		2	18	198 ± 65	76	<0.01
SHR	Harding-Passey melanoma	0	25	$1,860 \pm 170$	–	–
		2	25	$1,710 \pm 150$	8	>0.05

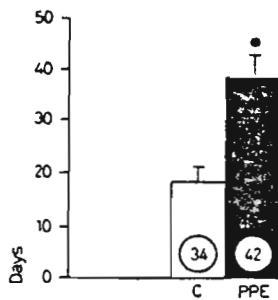


Fig. 1. Effect of PPE on the survival of mice inoculated with lympholeukemia LIO-1. Abscissa: C = Control (saline). Figures in columns = number of animals in the groups. * = The difference with control is significant, $p < 0.05$.

mice with transplantable lympholeukemia LIO-1 (fig. 1) but showed no effect on the survival time of (C57BL/6 × DBA/2) F_1 mice with leukemia L-1210 (7.8 ± 0.4 days for controls and 8.2 ± 0.2 days for treated mice).

(2) As can be seen from table II, PPE decreased the incidence of mammary adenocarcinoma induced with DMBA in rats. Adenocarcinomas of the mammary gland formed in 30 out of 37 DMBA-treated rats (81.1%), while DMBA and PPE administration was followed by the induction of adenocarcinomas in 9 out of 35 animals (25.7%). Thus, treatment with PPE resulted in the formation of fewer adenocarcinomas of the mammary gland. It should be pointed out that the latent periods of adenocarcinomas and fibroadeno-

mas were practically identical in the DMBA-treated rats, while in the DMBA- plus PPE-treated animals the latent periods of fibroadenomas were longer.

(3) As is shown by the data in table III, PPE treatment results in a longer life span of normal rats without any pretreatment; the life span of animals treated with 0.5 mg/day was longer than the average by 25%. These data, graphically presented in figure 2, clearly demonstrate the relationship of the life span extending effect of PPE and its dosage. Meanwhile, as the life span becomes longer, the number of tumor-bearing rats increases, although this rise is not statistically significant (table III). Since the frequency of spontaneous tumors, chiefly that of hormone-producing and hormone-dependent organs, increases with the prolongation of life span [2], it may be suggested that the increased incidence of spontaneous tumors in our experimental animals is determined by a longer life span. This conclusion is illustrated by the data presented in figure 3 on the yield of tumors in control and experimental groups. This conclusion is further confirmed by the findings showing that the animals' mean age, at which spontaneous tumors are revealed, is increased if PPE is administered. It should be emphasized that the rate of malignant tumor incidence both in control and experimental animals is the same, whereas the increase in the number of tumors per animal is mostly accounted for by such benign tumors as adenomas of the pituitary and mammary fibroadenomas.

As can be seen from the findings obtained, PPE exerted an anti-tumor effect in relation to 4 of 6 studied

Table II. Effect of PPE on the incidence of DMBA-induced mammary tumors in rats

Group	Num- ber of rats	Number of rats with tumors		Number of tumors		Mean latent period tumors days	Tumors of mammary glands						Other tumors			
		total	%	total	per rat		adenocarcinomas			fibroadenomas						
							number of rats	number of tumors	mean latent period days	number of rats	num- ber of tu- mors	mean latent period days				
DMBA + saline	37	36	97.3	52	1.44	102 ± 11	30	81.1	43	1.43	95 ± 6	3	8.1	3	81 ± 44	5 ¹
DMBA + PPE	35	28	80.0	33	1.18	$182 \pm 5^*$	9	25.7*	10	1.11	86 ± 9	12	34.3*	12	$216 \pm 21^*$	9 ²

¹ Cancer of Zymbal gland (2), leukemia (1), adenoma of thyroid (1), cancer of skin (1).

² Cancer of Zymbal gland (5), leukemia (4).

* The difference with control is significant, $p < 0.05$.

Table III. Incidence of tumors in female rats receiving PPE for 20 months

	Control (saline)	PPE	
		0.1 mg/day	0.5 mg/day
Number of rats surviving more than 6 months	75	39	33
Mean survival time, days	681 ± 14.5	749 ± 20.1*	852 ± 33.8*
Number (%) of rats with tumors	23 (30.7%)	13 (33.3%)	14 (42.4%)
Mean latent period, days	694 ± 22.6	811 ± 26.3*	868 ± 42.3*
Total number of tumors	30	22	27
Number (%) of rats with malignant tumors	7 (9.3%)	3 (7.7%)	3 (9.1%)
<i>Site and tumor type</i>			
<i>Pituitary</i>			
Adenoma	6	7	4
Adenocarcinoma	1	-	1
<i>Thyroid</i>			
Microfollicular adenoma	5	1	4
Clear-cell adenoma	3	2	2
<i>Adrenal cortex</i>			
Adenoma	2	-	1
<i>Mammary gland</i>			
Fibroma	1	1	1
Fibroadenoma	3	7	6
<i>Uterus</i>			
Polyp	-	-	3
Adenomyoma	1	-	-
Fibroadenoma	-	-	1
<i>Hematopoietic system</i>			
Thymoma	2	1	2
Leukemia	3	2	-
Lymphosarcoma	2	-	-
<i>Skin</i>			
Basalioma	-	-	1
<i>Soft tissues</i>			
Sarcoma	1	1	-
<i>Intestinum</i>			
Neurinoma	-	-	1

* The difference with control is significant, $p < 0.05$.

transplantable tumor strains. Besides, the administration of the PPE resulted in the decrease of incidence of DMBA-induced adenocarcinomas of the mammary gland. However, PPE did not show an effect on the incidence of spontaneous tumors in rats but increased the survival time of rats.

Discussion

At present, we cannot exactly designate the mechanism of the anti-tumor effect of PPE. Meanwhile, a number of suggestions can be made in this field.

The experiments carried out in this laboratory showed that transplantation of tumors caused diabetes-like changes which were revealed with intravenous glucose loading. The decrease in glucose tolerance was associated with hyperinsulinemia and hypercholesterolemia, that is, disturbances analogous to the patterns observed at early stages of adult onset diabetes mellitus. Besides, DMBA administration induced in rats the decrease of tolerance to glucose and the decrease of sensitivity to insulin [7]. Fatty acids, under these conditions, are the main energy substrate that, in turn, promotes the development of hypercholesterolemia and hypertriglyceridemia [19, 36]. We showed previously that PPE.

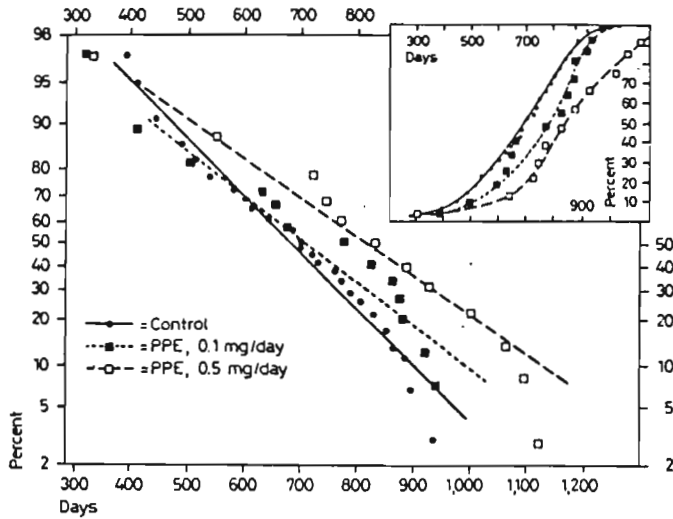


Fig. 2. Effect of PPE on the life span of rats. Ordinate axis = % (on the main diagram: probit scale); horizontal axis = days. Straight lines = survival; curves = mortality.

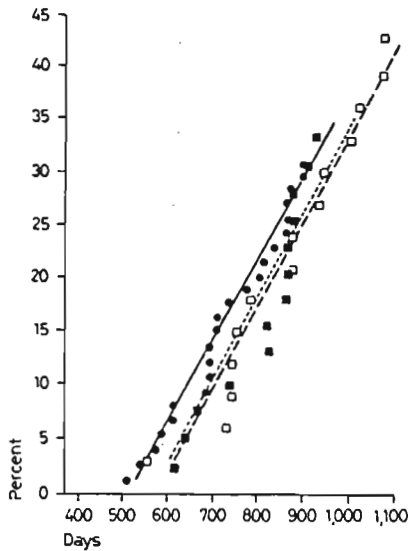


Fig. 3. Effect of PPE on the spontaneous tumor yield in female rats. Symbols as in figure 2.

obtained in this laboratory, caused in animals the increase of glucose utilization and decreased blood levels of immunoreactive insulin and triglycerides [41]. The mechanism of the anti-tumor effect of the pineal extract might be theoretically related to these influences on the fatty-carbohydrate metabolism.

Let us consider the data evidencing in favor of this suggestion. As for hyperinsulinemia, it should be taken

into consideration that insulin stimulates the division of both normal somatic cells [48] and tumor cells, e.g., mammary cancer cells [25]. Fatty acids also have the ability to stimulate the division of somatic cells, including that of tumor ones [26]. Of significant importance is the role of hypercholesterolemia as the factor which is required for the division of non-lymphoid cells. This ability of cholesterol is connected with the role it plays in the structure of the plasmatic membrane. In particular, it was shown that derivatives of cholesterol which inhibited cholesterol synthesis produced the arrest of the growth of fibroblasts in tissue culture [14]. The synthesis of cholesterol is intensified in fibroblasts transformed by SV-40 virus [27] as well as in some tumors induced by various carcinogens [46].

Thus, there are good reasons to believe that increased blood levels of insulin and cholesterol (or, more exactly, lipoproteins of low density) are the factors stimulating the division of both normal non-lymphoid as well as tumor cells. On the other hand, the same factors show a reverse, that is, inhibiting action on the functional activity of thymus-dependent lymphocytes and macrophages [18, 19]. In particular, it was shown that the enrichment of the lymphocytic membrane with cholesterol suppressed the blastogenic response of lymphocytes to some mitogens [1].

The decrease of cell-mediated immune activity caused by metabolic factors was designated by Dilman [18, 19] as metabolic immunodepression. Thus, the same metabolic factors, on the one hand, stimulate the division of non-lymphoid cells and conversely cause metabolic immunodepression on the other. These opposite effects form the syndrome of cancrophilia [17-19], that is, a number of metabolic conditions promoting both tumor development and progression. From this point of view the anti-tumor effect of PPE could be connected, at least theoretically, with the improvement of the metabolic pattern. It should be stressed in this respect that PPE exerted a stimulating action on the immune system [10, 20]. Therefore, the following hypothesis on the mechanism of the anti-tumor effect of PPE may be advanced: PPE was shown to inhibit a number of transplantable tumors, i.e., under the conditions when tumor transplantation induced disturbances characteristic of the syndrome of cancrophilia. Moreover, PPE administration reduced the frequency of DMBA-induced mammary adenocarcinomas. At the same time, it was shown that DMBA treatment results in the development of metabolic shifts, typical of cancrophilia syndrome [7]. Taking into consideration the

ability of PPE to alleviate metabolic shifts and, especially, to improve carbohydrate tolerance, to reduce blood insulin and triglyceride levels and, therefore, to improve cellular immunity [10, 20], it may be supposed that PPE exerts an anti-tumor effect whenever the development and progression of the tumor process contribute to cancrophilia. On the other hand, it may be suggested that a benign tumor cannot induce such shifts as are observed in cancrophilia syndrome. In such cases the anti-tumor effect of PPE is not likely to take place. In this connection it should be mentioned that PPE administration to DMBA-treated rats resulted in a lower incidence of malignant neoplasms set off by an increase in the frequency of fibroadenomas which occur at a relatively later period of the animal's life span (table II).

Moreover, a number of other aspects related to this problem should be taken into consideration. Thus, in particular the experiments carried out in this laboratory showed that administration of DMBA to rats resulted in the elevation of the hypothalamic threshold to suppression by estrogens, decreased tolerance to glucose and decreased sensitivity to insulin action [5, 7]. All similar disturbances can be corrected to a large extent by PPE [3, 6, 39]. Finally, the results obtained by us in collaboration with *M.N. Kondrashova* showed that PPE improved functional activity of mitochondria [31].

Thus, not excluding any other possible mechanisms of the anti-tumor effect of PPE, we should like to emphasize that the ability of the pineal gland to increase the sensitivity of the hypothalamic-pituitary system to regulating stimuli and to eliminate metabolic immunodepression and cancrophilia must be taken into consideration in the evaluation of the problem.

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V.M. Dilman, Laboratory of Endocrinology, Petrov Research Institute of Oncology, 68 Leningradskaya St., Pesochny-2, Leningrad 188646 (USSR)