Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Effect of isologous pineal transplants on tumorigenesis of autologous intrasplenic ovarian transplants in Swiss mice

Trimbak Chapekar

Department of Biochemistry, All India Institute of Medical, Sciences,
New Delhi - 110029, India

Correspondence to: Trimbak Chapekar, Ph.D., 1575 A, Sadashiv, Tilak Road,
Pune - 411030, India
TEL: +0091 20 433 3476

Submitted: *May 20, 1999*
Accepted: *August 11, 1999*

Key words: **pineal; intrasplenic ovary; tumorigenesis**

*Neuroendocrinology Letters 1999; **20**:305–309 pii: NEL200599A03*

Abstract

Intrasplenic ovarian tumorigenesis is the most suitable experimental model to study the role of hormones in carcinogenesis. If increased secretion of gonadotrophins causes ovarian tumorigenesis in the spleen, one can find ways to control the secretion of gonadotrophins so as to prevent the tumor formation. Pineal is the one gland which is known to regulate gonadotrophin secretion. In the present studies, isologous pineals were transplanted into the anterior eye chamber of Swiss females bearing autologous ovaries in the spleen, with appropriate controls. The results showed that two pineals maintained in the ocular chamber for four months could prevent tumor formation of the ovarian transplants in the spleen. Control animals maintained for the same period of time as experimental animals had ovarian transplants turn into a tumor. These experiments clearly demonstrated that the pineal had an inhibitory effect on intrasplenic ovarian tumorigenesis. Since two pineals were required to bring about the effect, it implies that the amount of the factor present in the endogenous gland is not enough to prevent the tumorigenesis. The inhibitory action in these experiments must be via the pituitary, since it is not inhibition of tumor growth but it is prevention of transformation of the normal ovary into a tumor. This prevention is possible only if hormone stress is withdrawn from the system. These results suggest the prospects of prevention of cancer by some factor present in the pineal.

Introduction

Intrasplenic ovarian tumorigenesis, an experimental model, first developed by Biskind and Biskind [1], was shown to be the consequence of increased gonadotrophin secretion in the animal [2]. A question was, therefore, asked if the tumorigenesis of the intrasplenic ovary could be prevented by the factor(s) that control(s) the secretion of pituitary gonadotrophins. The pineal is known to have a regulatory effect on the gonadotrophin secretion [3, 4, 5]. Pineal extracts have been shown to inhibit the effects of both exogenous and endogenous gonadotrophins [6]. Further, melatonin free pineal extracts have been shown to have gonadotrophin inhibiting activity [7, 8].

Hormones have been shown to be involved in the genesis and growth of neoplasia [9, 10, 11]. Sustained high levels of hormones exert a physiological stress over the target cells which react to it, transforming into neoplasia. Controlled levels of hormones, therefore, may reduce the risk of development or progression of cancer. In the present studies, the effect of additional isologous pineal glands on the tumorigenesis of ovary in the spleen has been investigated.

Materials and Methods

Forty-day-old Swiss females were used for the intrasplenic transplantation of the autologous ovary. Pineals were collected from 60-day-old Swiss males.

Female mice were divided into three groups:

1. Experimental Group: Intrasplenic ovarian transplants with intraocular pineal transplants; 2. Control Group I: Intrasplenic ovarian transplants; and 3. Control Group II (sham control): Intrasplenic ovarian transplants with liver pieces into the ocular chamber.

Transplantation of the ovary into the spleen:

Animals were anesthetized with sodium barbiturate (300 ug/10 gm body weight) to perform bilateral ovariectomy and subsequent transplantation of an ovary into the spleen. One of the two ovaries was cut into pieces of about 0.8 mm size and transplanted autologously into the spleen with the help of a sharp hypodermic needle (21 guage, 1.5 inches length). The other ovary was discarded. A total of twenty animals were so transplanted, out of which ten were included in the experimental group while five were in each of the two control groups.

Intraocular transplantation of pineals:

A day after the ovary was transplanted into the spleen, pineals from 60-day-old males were transplanted into the anterior eye chamber of these animals by the method described earlier [12]. A total of ten mice were transplanted with two pineals per animal.

Control Groups:

Two control groups were maintained: Control Group I consisted of five animals with intrasplenic ovary only. Control Group II consisted of five animals with intrasplenic ovary and liver pieces in the anterior eye chamber serving as sham controls.

All the mice were killed after four months of transplantation. Ovary and pineal transplants were retrieved and fixed in Bouin's fixative. The sections of the tissues were stained with hematoxylin and eosin.

Results

1. Forty-day-old ovary:

The ovary of a 40-day-old Swiss mouse has a few Graafian follicles, a few secondary follicles and many primary follicles (Fig. 1). Mitotic activity in granulosa cells of growing follicles

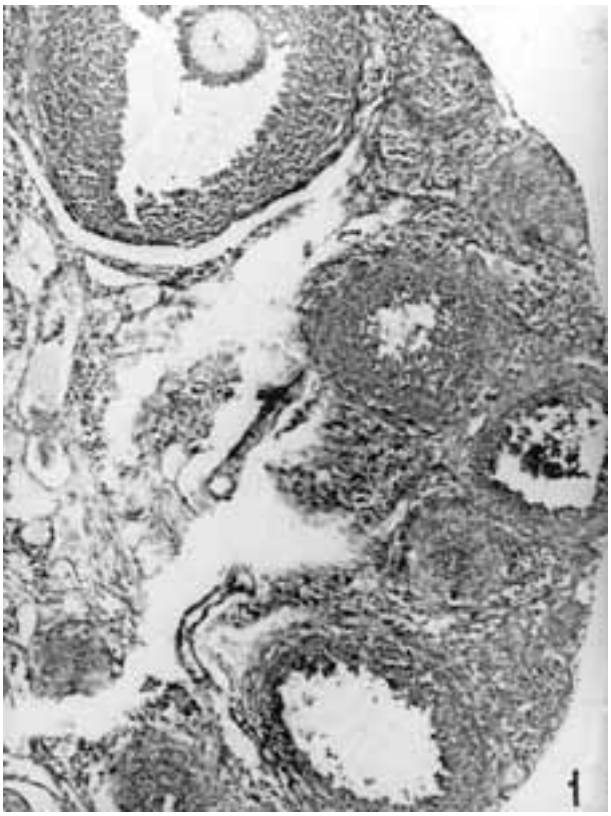

Fig. 1. Ovary of a 40-day-old Swiss mouse showing follicles at varying stages of differentiation. Mostly they are secondary follicles. Stromal tissue (thecal cells) is scanty.

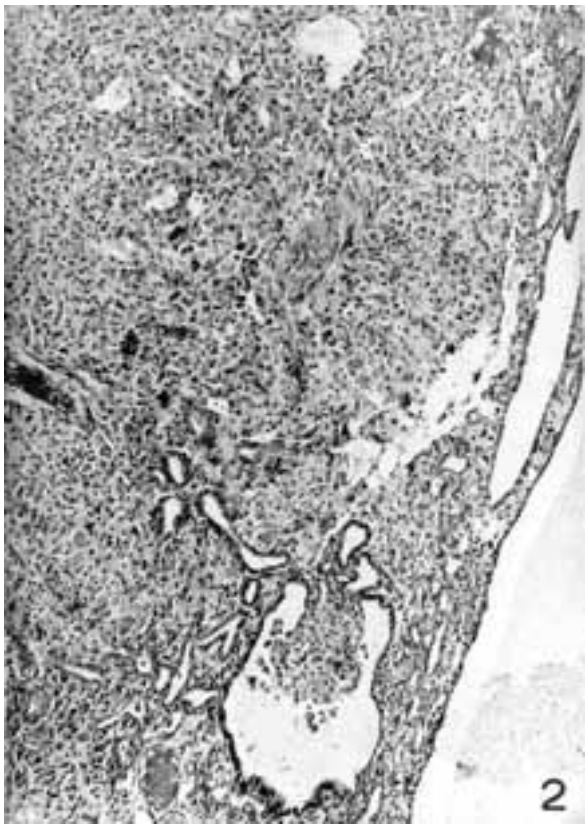

Fig. 2. Intrasplenic ovarian transplant maintained for four months. The transplant has turned tumoral.

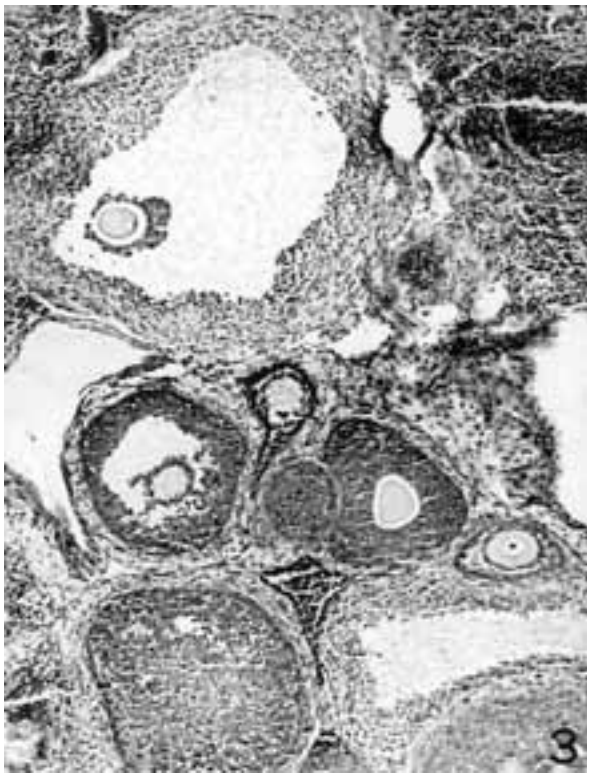

Fig. 3. Intrasplenic ovarian transplant maintained for four months. (The animal also received two pineals from male Swiss mice in the ocular chamber.) The ovarian transplant has not turned tumoral. The normal ovarian organization is evident. Thecal cells, however, appear to have proliferated a few layers.

was appreciable (about 10–12 mitoses per growing follicle). Thecal layer was very scanty.

2. Intrasplenic ovary:

The 4-month-old ovarian transplants in the spleen in all the five animals turned tumoral. (Fig. 2). The normal morphology of the ovary was replaced by a mass of thecal cells and glandular tissue consisting of primary and secondary follicles. The average greater diameter of the tumors was 4.12 mm.

3. Intrasplenic ovary (with liver grafts):

This group was a sham control. The liver grafts apparently had no interfering effect on the tu-morigenesis of the intrasplenic ovarian transplants which grew into a tumor. The average greater diameter of the tumors was 4.0 mm. The tumor mass was predominantly formed of thecal cells.

4. Intrasplenic ovary (with two pineal grafts):

The ovarian transplants in all the ten animals in this experimental group did not turn tumoral (Fig. 3). They showed normal morphology of follicles although thecal cells proliferated to a few layers. Mitoses were observed in granulosa cells of growing follicles.

Histological sections of pineal grafts showed that the grafts were viable in the ocular chamber (Fig. 4).

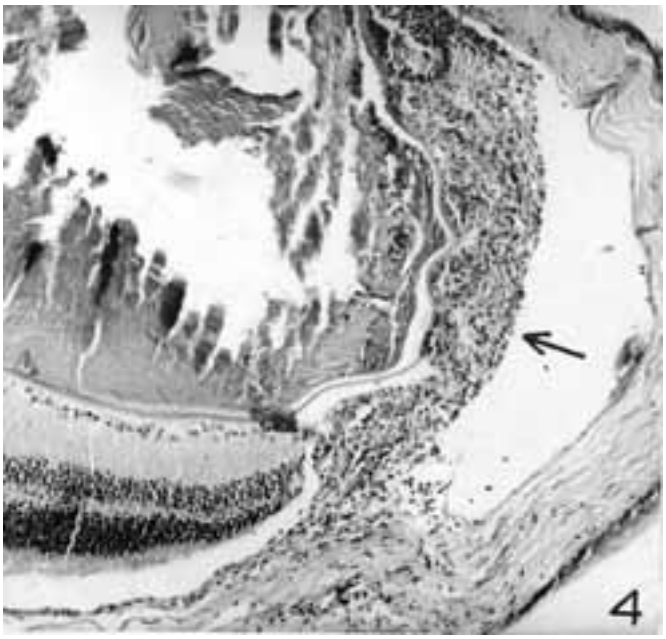

Fig. 4. Pineal transplant in the ocular chamber. The transplant is indicated by an arrow. Two pineals from male Swiss mice were grafted. Apparently, they fused to form one transplant.

Discussion

The pineal gland has been shown to have a regulatory effect on a wide range of physiological processes [13, 14]. Besides melatonin, serotonin and their derivatives, the gland synthesizes certain polypeptides [15, 16]. These products have been shown to inhibit secretion of anterior pituitary hormones including gonadotrophins. The gland has been shown in a variety of systems to have a direct inhibitory effect on mitosis [17], and so also on tumor growth [18]. It also appears from the reports that the neuroendocrine gland is not required to be in its normal location to be functional. The chick embryonic pineal gland has been shown to be functionally active in organ culture [19]. Shein also has shown that noradrenaline stimulated rat pineal organ cultures synthesized melatonin from 14C tryptophan [20].

In the present report, the effect of additional pineals on the tumorigenesis of intrasplenic ovarian transplants has been studied. We have earlier reported that autologous ovaries in the spleen of Swiss mice developed into a tumor after about three months of transplantation [21]. The experiments presented herein have demonstrated that the intrasplenic ovarian transplants did not develop into a tumor during the period of four months when the animals bore two pineals in the ocular chamber.

We grafted varying numbers of pineals like one, two and three per animal. The response of ovarian transplants to two and three pineals was similar. One pineal graft per animal did not elicit an inhibitory response. These two groups—one pineal per animal, and three pineals per animal—are not included in this report. The results of two pineals per animal are reported herein.

The consideration to use male donors for pineals was that we wanted to spare females for transplantation of the ovary.

The present experiments clearly demonstrated that the pineal had an inhibitory effect on intrasplenic ovarian tumorigenesis. It also suggests that the animal's endogenous pineal is not sufficient to prevent the tumorigenesis. It thus appears that the pineal factor that brings about inhibition is required to be in a greater amount than it is present in the pineal of the animal. This is met by two pineal transplants.

The mechanistic explanation for the action of pineal transplants on ovarian tumorigenesis is not possible with these experiments. The action can be either direct on the transplants or via the pituitary. However, we conjecture that the inhibitory action in this case is via the pituitary, since it is not the inhibition of tumor growth but it is prevention of the transformation of a normal ovary into a tumor. This prevention is possible only if hormone stress is withdrawn from the system.

The present experiments are, in this way, different. The effect of the pineal has been observed on the development of a tumor and not on tumor growth. This experimental system is used perhaps for the first time to demonstrate the pineal effect on tumorigenesis. (An extensive literature search did not reveal any reports using this system. Nor are there related reports in recent years.) These results suggest the prospects of prevention of cancer by some factor present in the pineal.

These experiments may prove to be supplementary to the earlier observations on the anticancer activity of pineal factors [22].

Acknowledgments

The author greatly appreciates the peripheral technical assistance rendered by Mr. K. K. Sarin. Thanks also to Mrs. Snehal Bhalerao for her meticulous typing and to Dr. Madhavarao for the final layout and printing of the manuscript.

REFERENCES

1 Biskind MS, Biskind GR. Development of tumors in rat ovary after transplantation into the spleen. Proc Soc Exp Biol Med 1944; **55**:176.
2 Li MH, Gardner WU. Experimental studies on the pathogenesis and histogenesis of ovarian tumors in mice. Cancer Res 1947; **7**:549–66.
3 Wurtman RJ, Altschule MD, Holmgren U. Effects of pinealectomy and of a bovine pineal extract in rats. American J Physiology 1959; **197**:108–10.
4 Wurtman RJ, Axelrod J, Chu EW. Melatonin, a pineal substance: Effect on the rat ovary. Science 1963; **141**:277–78.
5 Motta M, Fraschini F, Martini L. Endocrine effects of pineal gland and of melatonin. Proc Soc Exp Biol Med 1967; **126**:431–35.
6 Cheesman DW, Forsham PH. Inhibition of induced ovulation by a highly purified extract of the bovine pineal gland. Proc Soc Exp Biol Med 1974; **146**:722–24.
7 Orts RJ, Benson B. Inhibitory effects on serum and pituitary LH by a melatonin free extract of bovine pineal glands. Life Sciences, Part II 1973; **12**:513–19.
8 Damian E, Ianas O, Badescu I, Oprescu M. Gonadotropin inhibiting activity of melatonin-free pineal extract. In: Birau M, Schloot W, editors. Melatonin: Current Status and Perspectives. Pergamon Press 1981. p. 171–75.
9 Poel WE. The cause and nature of cancer. In: Hamberger F, editor. Prog Exp Tumor Res. Vol. 5, New York: Hafner Publishing Co. 1964. p. 53–84.
10 Huggins C. Control of cancers of man by endocrinologic methods. Cancer Res 1957; **17**:467–72

11 Furth J. Hormones as etiological agents in neoplasia. In: Becker F, editor. Cancer Vol I Plenum Press 1975. p. 75–120.
12 Chapekar TN, Nayak GV, Ranadive Kamal J. Studies on the functional activity of organotypically cultured mouse ovary. J Embryol Exp Morph 1966; **15**:133–41.
13 Vaughan MK, Johnson LY, Blask DE, Reiter RS. Melatonin stimulation of prolactin secretion in male rats: Influence of pinealectomy, castration and gonadal steroid pretreatment. In: Birau N, Schloot W, editors. Melatonin: Current status and perspectives. Pergamon Press; 1981. p.165–70.
14 Provinciali M, Di Stefano G,Bulian D,Tibaldi A, Fabri SN. Effect of melatonin and pineal grafting on thymocyte apoptosis in ageing mice. Mech Ageing Dev 1996; **90**(1):1–19.
15 Reiter RJ, Vaughan MK. Pineal antigonadotrophic substances: Polypeptides and indoles (Minireview). Life Sciences 1977; **21**:159–72.
16 Noteborn HPJM, Bartsch H, Bartsch C, Mans DRA, Weusten JJAM, Fletinig B, et al. Partial purification of (a) low-molecular weight ovine pineal compound(s) with an inhibiting effect on the growth of human melanoma cells in vitro. J Neural Transm 1988; **73**(2):135–55.
17 Bindoni M, Jutisz M, Ribot G. Characterisation and partial purification of a substance in the pineal gland which inhibits cell multiplication in vitro. Biochim Biophys Acta 1976; **437**:577–88.
18 Hamilton T. Influence of environmental light and melatonin upon mammary tumour induction. Brit J Surg 1969; **56**:764–66.
19 Mezei C, Wainwright SD. Hormone-induced increase of hydroxy indole-o-methyl tranferase activity in the embryonic chick pineal gland in organ culture. Life Sciences 1979; **24**:1111–18.
20 Shein HM. Control of melatonin synthesis by noradrenaline in rat pineal organ cultures. In: Wolstenholme GEW, Knight J, editors. The Pineal Gland. Edinburgh and London: Churchill and Livingstone 1971. p. 197–212.
21 Chapekar TN. Tumorigenesis of autologous intrasplenic ovarian transplants in Swiss mice. Ind J Med Res 1982; 76:565–70.
22 Lissoni P, Brivio O, Brivio F, Barni S, Tancini G, Crippa D, et al. Adjuvant therapy with the pineal hormone melatonin in patients with lymph node release due to malignant melanoma. J Pineal Res 1996; **21**:239–42.