Inhibition of Prolactin-induced Mammary Cancer in C3Hf (XVII) Mice with the trans Isomer of Bromotriphenylethylene

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ABSTRACT

C3Hf (XVII) mice never develop spontaneous mammary tumors. However, the transplantation of an isologous pituitary gland under their kidney capsule is followed by a 10-fold increase in serum and pituitary prolactin content (180 ng/ml and 20 μg/mg of tissue, respectively), concomitant with an increase of prolactin receptors in mammary glands. Under these conditions, mammary tumors appear in 90% of the mice.

If a racemic brominated triphenylethylene, i.e., broparestrol, is administered, serum and pituitary prolactin decrease rapidly (10 ng/ml and 4 μg/mg of tissue, respectively), and prolactin receptors in the mammary gland are markedly reduced. This compound also inhibits the development of normal mammary glands, prevents mammary carcinogenesis, and unexpectedly causes a significant atrophy of the ovaries.

Our study confirms that prolactin is a key hormone involved in murine mammary carcinogenesis and that it can act directly on the mammary gland by stimulating the level of its own receptor.

INTRODUCTION

Prolactin plays a major role in the etiology of mammary tumors in rodents (3, 24, 31, 47). It is now established that a high blood level of prolactin produces, with great frequency, adenocarcinomas in mice of every strain, even if the animals do not harbor the mammary tumor agent (24). Such a high level can be induced by grafting an isologous pituitary under the kidney capsule, as was first demonstrated by Desclin (9). It was further demonstrated (49) that drug-induced suppression of prolactin secretion in pituitary isograft-bearing mice resulted in a sharp reduction in the incidence of mammary tumors. It appeared also that there was a clear correlation between the concentration of serum prolactin and spontaneous mammary carcinogenesis in control females. High-mammary-cancer C3H mice have an elevated level of prolactin, whereas low-mammary-cancer C57BL mice have a decreased level at 250 days (40).

The demonstration that prolactin is one of the major factors in the etiology of mammary cancer in rodents initiated a great amount of research on antiprolactin compounds of various chemical structures (8, 17, 23, 25, 26, 29, 30, 41, 43, 46). The C3Hf (XVII) females which were used in our experiments never developed any spontaneous mammary tumors. However, following the transplantation of an isologous pituitary gland under the kidney capsule, 90% presented such cancers (33). It was shown recently in life span experiments that a brominated triphenylethylene, commonly known as broparestrol, administered to pituitary-transplanted young C3Hf (XVII) females, inhibits the development of mammary glands (22) and of mammary carcinogenesis (33). Broparestrol presents 2 isomer forms like clomiphene, another derivative of triphenylethylene, of which both isomers have been reported to produce different reactions in experimental animals (15, 16). Accordingly, we decided that it would be interesting to study separately the biological activities of the trans and the cis forms (Chart 1).

In the present work, we will report some of our results obtained with the trans isomer, TBP.3

MATERIALS AND METHODS

Two hundred sixty C3Hf (XVII) females (colony of the Curie Fondation), 25 to 30 days old, were used for the biological experiments. An isologous male pituitary gland transplantation under the left kidney capsule was performed on all of them. Fourteen mice received no other treatment. Seven mice were sacrificed on the 25th day. Fourteen mice received a diet containing 2 ppm (7.5 μg TBP per mouse per day), 14 received a 20-ppm diet, and 14 others received a diet containing 200 ppm of the compound (22). Seven more were sacrificed on the 25th day, and the remaining 7 mice were sacrificed on the 50th day. A complete autopsy was performed on all the animals. Furthermore, the 4th left mammary gland from each mouse was prepared for a whole-mount dissection (22).

The 204 remaining mice were divided into 4 groups and received the same diets that the 56 others received, i.e., normal diet or diets containing 2, 20, or 200 ppm of the drug, and were observed in a life span experiment.

Most of the mice were sacrificed when moribund. Spontaneous death was exceptional. An autopsy was performed on each of them. Mammary tumors, grafted and in situ pituitary glands, adrenals, ovaries, uterine horns, and occasional pathological findings such as liver lesions or pulmonary tumors were fixed in Bouin-Holland fluid for a routine microscopic examination after hematoxylin-eosin-safran staining.

The biochemical experiments were carried out on 175 females at 30 days of age. The mice were divided into 16 groups as shown in Table 1. They were killed by cervical dislocation; blood was collected by cardiac puncture and allowed to clot at 4°C. Serum was separated and stored at −20°C until use. One mammary gland and the pituitary from each mouse were immediately excised, weighed, and stored at −20°C.

Highly purified bovine prolactin (NIH-P.B. 4) was used for binding studies. Dr. N. Y. Sinha supplied us with the reagents.


2 To whom requests for reprints should be addressed.

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3 The abbreviation used is: TBP, trans-broparestrol.
for radioimmunoassay of mouse prolactin. For iodination of prolactin, carrier-free Na\(^{125}\)I was obtained from the Radiochemical Centre, Amersham, England; lactoperoxidase was obtained from Calbiochem, Los Angeles, Calif., and diluted peroxide was from Mallinckrodt Chemical Works, St. Louis, Mo.

Hormone Assay. The prolactin content of the pituitary glands and sera was measured by specific homologous radioimmunoassay. For iodination of prolactin, carrier-free Na\(^{125}\)I was obtained from the Radiochemical Centre, Amersham, England; lactoperoxidase was obtained from Calbiochem, Los Angeles, Calif., and diluted peroxide was from Mallinckrodt Chemical Works, St. Louis, Mo.

Preparation of Tissue Particles. The fourth abdominal mammary glands were quickly excised and chilled. For the preparation of a crude low-speed pellet, the method of Turkington (37) was used. Highly purified cell membrane fractions were prepared by sequential centrifugation at 15,000 and 100,000 x g (37). The 100,000 x g pellet was resuspended in 2.5 ml Tris-HCl buffer (pH 7.4) containing 10 mM CaCl\(_2\) and 0.1% bovine serum albumin, and the protein concentration was estimated according to the method of Lowry et al. (20). The specific binding of \(^{125}\)I-labeled prolactin was determined as follows. To 100 \(\mu\)l of the membrane preparation containing 100 to 300 \(\mu\)g of protein were added 100 \(\mu\)l of unlabeled bovine prolactin (10 \(\mu\)g/ml solution), or 100 \(\mu\)l of buffer and 100 \(\mu\)l of \(^{125}\)I-labeled prolactin (150,000 dpm = 1 ng, approximately).

The tubes were incubated for 2 hr at 25° or for 24 hr at 4°, according to the methods of Sakai et al. (34) and Powell et al. (28). Scatchard analyses (35) were carried out with increasing concentrations of \(^{125}\)I-labeled prolactin in the presence or absence of a 2,000-fold excess of unlabeled bovine prolactin (the molecular weight of prolactin was assumed to be 23,000). Cold buffer (3 ml) was added to each tube, and the membranes were precipitated by centrifugation at 2,000 x g for 20 min at 4° and then counted in a Packard Tri-Carb scintillator with a 30% efficiency. All tissues from 4 to 10 animals were pooled, and results included in the paper are based on duplicate determinations.

RESULTS

As shown in Figs. 2 to 4, the development of the mammary glands is strongly inhibited by the 2 high doses, and to a lower degree by the dose which corresponds to a daily intake of 6 to 8 \(\mu\)g/mouse. The inhibition appears even after a short time, i.e., 25 days, and seems more pronounced after 50 days. In fact, these results are similar to those obtained with the trans-cis mixture (22). Administration of TBP in life span experiments produced particularly interesting results. As could be expected, the 2 high doses suppressed completely mammary carcinogenesis, as was the case with broparestrol (33). However, it should be remembered that this last compound, incorporated in the diet at a level of 2 ppm, did not prevent the tumor appearance. At the same dose, the trans isomer produced a nearly total inhibition. Only one tumor was observed in a group of 54 mice (Table 2). It appears in Table 3 that the pituitary isograft produced a hypertrophy of the ovaries, probably due to the development of several corpora lutea. In mice treated

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**Chart 1. Structure of cis- and trans-broparestrol (bromotriphenylethylene).**

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**Table 1**

<table>
<thead>
<tr>
<th>Groups of mice used for biochemical experiments</th>
<th>No. of mice</th>
<th>TBP (20 ppm) in diet</th>
<th>Days after TBP treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>7</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>130</td>
</tr>
<tr>
<td>Pituitary isograft-bearing mice</td>
<td>7</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>-</td>
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<td>-</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>125</td>
</tr>
</tbody>
</table>

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**Table 2**

<table>
<thead>
<tr>
<th>Mammary carcinogenesis of female C3H/HeJ mice bearing a transplanted pituitary and receiving TBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eagle</td>
</tr>
<tr>
<td>No. of mice</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>54</td>
</tr>
<tr>
<td>75</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Weight of different glands of female C3H/HeJ mice bearing a transplanted pituitary and receiving TBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eagle</td>
</tr>
<tr>
<td>Ovaries (mg)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>2 ppm</td>
</tr>
<tr>
<td>20 ppm</td>
</tr>
<tr>
<td>200 ppm</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* Numbers in parentheses, number of mice.
* Nonsignificant versus control.
* p < 0.01 versus controls.
* p < 0.05 versus controls.

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Inhibition of Mammary Carcinogenesis in Mice
with TBP, the weights of the ovaries remained similar to those usually observed in 2- to 3-month-old mice. It is well known that ovaries of such young females do not secrete progesterone.

The biochemical investigations on the receptors in mammary glands revealed that their level was in all cases too low to be detected in crude particulate extracts. Therefore, we used only membrane fractions to determine the binding capacity. Scatchard analyses gave an apparent Kd of approximately 10^-9 M.

Chart 2 shows that the level of circulating prolactin increases gradually in grafted mice as compared to the nongrafted animals. In all cases, treatment with TBP results in a significant reduction of serum prolactin.

Chart 3 shows the same patterns for pituitary secretion of prolactin as those obtained for serum prolactin, indicating that the graft induces a continuous secretion of the hormone and that TBP administration suppresses it.

Chart 4 shows that after 20 days the specific binding of prolactin in mammary glands is more important in grafted mice than in all the other groups. TBP treatment induces a significant decrease in binding both in control and in grafted mice.

As shown by Chart 5, the binding capacity at 25° is more important as a result of the exchange procedure that occurred at this temperature. Nevertheless, the overall patterns remain similar, indicating an increase in prolactin binding in the grafted animals and a decrease as a result of TBP treatment.

**DISCUSSION**

TBP is the trans isomer of broparestrol and is a powerful inhibitor of the development of mammary glands and of mammary carcinogenesis in female C3Hf(XVII) mice bearing a transplanted pituitary under the kidney capsule. The inhibitions proceed through the depression of prolactin secretion.

Studies of hormonal control by prolactin have been performed on the liver (7, 21), rabbit mammary gland (11), 7,12-dimethylbenzanthracene-induced mammary tumors (10), and murine mammary gland (36). We extended these studies to the C3Hf(XVII) experimental model and showed that the pituitary isograft enhanced pituitary and serum prolactin concentration, along with an increase of specific binding of 125I-labeled bovine prolactin to mammary glands. TBP brought down the levels of
circulating prolactin concurrent with reduced specific binding. It thus appears that an increase in serum prolactin raises its own specific binding to the target tissue and consequently increases mammary gland development and mammary carcinogenesis. Induction of prolactin receptors by prolactin itself in liver tissues has been demonstrated by transplanting a pituitary under the kidney capsule (27) or by exogenous treatment with prolactin (7). It has been shown that low doses of estrogens increase prolactin binding as well as pituitary and serum prolactin (5, 36) and that ovariectomy causes a significant decrease in prolactin binding to liver tissue (14). It is known that mammary growth and mammary carcinogenesis are very susceptible to the hormonal environment, since most hormone-responsive mammary tumors in rodents appear to require the participation of both the pituitary glands and the ovaries for an optimal growth process.

Our studies revealed that TBP treatment causes the atrophy of the ovaries associated with a reduced prolactin level. It has been shown that treatment with several alkaloids leads to a regression of the ovaries (4, 39, 48, 50). The significance of such a regression remains unclear, although it has been suggested that this effect could be due to an inhibition of luteal activity by pituitary prolactin (45).

It has been shown recently that concurrent treatment with TBP produced a strong inhibition of induced carcinogenesis of 7,12-dimethylbenzanthracene-treated Sprague-Dawley female rats. However, it was also observed that the ovaries of the females which were fed a normal diet contain a great number of large corpora lutea. No such structures developed in the ovaries of animals fed a diet containing 2 or 20 ppm of TBP (32).

It is possible that the cancer-inducing action of prolactin, as well as the inhibition obtained with antiprogestin compounds, proceed finally from the influence of this pituitary hormone on the secretions of the corpus luteum or on the inactivation of circulating progesterone.

Astwood (1) was the first one to show in 1941 that, among other functions, prolactin maintains the secretory activity of corpus luteum, and more recently, Behrman et al. (2) have established that prolactin promotes the secretion of progesterone. Swearingen and Nicoli (42) have shown that prolactin is an inhibitor of the synthesis of the 20-α-hydroxysteroid dehydrogenase which converts progesterone into an inactive metabolite, 20-α-hydroxy-4-pregnen-3-one. It can be postulated that antiprogestin compounds, such as TBP, favor the synthesis of the enzyme, and accordingly inactivate progesterone.

These experiments have demonstrated that, at least in rodents, the presence or the absence of prolactin in the circulation plays an important role in induced mammary carcinogenesis of pituitary-transplanted mice or dimethylbenzanthracene-treated rats. Furthermore, it is probable that this hormone intervenes also in the etiology of spontaneous mammary carcinogenesis (40).

It has not yet been clearly established what mechanism makes prolactin a cancer-inducing agent. Several investigations suggest that estrogen-induced cancers have to be attributed to a rise of prolactin secretion after administration of the ovarian hormone (5, 18, 19).

Several chemical compounds, such as lysergide, L-dopa, bromocryptine, pergynine, and others are inhibitors of mammary carcinogenesis in rats. In nearly all cases, the chemical is a depressor of serum prolactin by means of a hypothalamic-pituitary inhibition (12), probably through its direct action on the prolactin-inhibiting factor or on the prolactin-releasing factor of the hypothalamus (6).

ACKNOWLEDGMENTS

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alkaloids and promotion of tumorigenesis by pituitary isografts in adreno-
Figs. 1 to 4. Mammary glands of 75-day-old C3Hf (XVII) females which bore a pituitary isograft during 50 days. × 18.

Fig. 1. Control without treatment.
Fig. 2. Female on a 2-ppm TBP diet for 50 days.
Fig. 3. Female on a 20-ppm TBP diet for 50 days.
Fig. 4. Female on a 200-ppm TBP diet for 50 days.
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