The Conversion of an Ovariectomy-nonresponsive to an Ovariectomy-responsive Mammary Tumor Strain

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SUMMARY

MTW9, a transplantable mammary tumor in Wistar Furth rats, shows little growth unless serum prolactin is increased. This study compares the response to ovariectomy of MTW9-MtT, a tumor developed in rats bearing the mammosomatotropic tumor MTW10 (serum prolactin 500 to 7000 ng/ml) with MTW9-P developed in rats given chronic perphenazine treatment (4 mg/kg/day). Serum prolactin concentrations were 150 to 600 ng/ml in MTW9-P-bearing rats. MTW9-MtT does not regress after ovariectomy but does regress after surgical removal (resection) of MtT. Ovariectomy plus MtT resection leads to greater tumor regression than MtT resection alone.

MTW9-P does not regress when perphenazine administration is stopped but does regress after ovariectomy, whether or not rats are given perphenazine. Administration of estradiol (10 µg/day) to rats with complete ovariectomy-induced regression of MTW9-P results in regrowth of tumor.

These data suggest that MTW9-P may represent a clone of MTW9 with a lower requirement for prolactin.

INTRODUCTION

MTW9 is a prolactin-dependent mammary adenocarcinoma developed in W/Fu rats (7, 8). When MTW9 is transplanted into syngeneic animals, it shows little growth unless serum prolactin is elevated. High serum prolactin concentrations can be easily achieved by coimplantation of a mammosomatotropic tumor, which produces growth hormone and prolactin (5). Growth of MTW9 can also be stimulated if estradiol (10 µg/day) is given to rats with complete ovariectomy-induced regression of MTW9-P resulting in regrowth of tumor.

Administration of perphenazine (4-[3-(2-chlorophenothiazin-10-yl)propyl]-1-piperazine ethanol) also resulted in elevated levels of serum prolactin (1). Pearson et al. (13) showed that chronic perphenazine administration increased serum prolactin concentration and the growth rate of dimethylbenz(a)anthracene-induced carcinomas. Haloperidol (4-[4-p-chlorophenyl]-4-hydroxypiperidino)-4'-fluorobutyrophene) and methyldopa, 2 drugs that elevate serum prolactin, have also been shown to enhance mammary tumor growth in rats (14, 15). Bogden et al. (2) demonstrated that perphenazine administration caused a dose-related increase in serum prolactin concomitant with increased growth of 13762, a MT transplantable to Fischer rats.

The present study compares MTW9 grown in rats bearing MTW10 with rats given chronic perphenazine treatment. MTW9 obtained by daily injections of perphenazine regresses after ovariectomy. MTW9-MtT, the MT obtained by coimplantation of MTW10, does not.

MATERIALS AND METHODS

Animal and Transplantation Procedures. W/Fu female rats were obtained from the Mammalian Genetics and Animal Production Section, Drug Research and Development, of the National Cancer Institute, and from Microbiological Associates, Walkersville, Md. Mammary tumor MTW9, supported by mammosomatotropic tumor MtTW10, was obtained from Dr. U. Kim, Roswell Park Memorial Institute, Buffalo, N. Y.

MTW9 was transplanted in W/Fu rats weighing about 150 g. The MT was minced with an equal volume of the Mixture of prolactin, growth hormone, estradiol, and progesterone (9).

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MTW9, a transplantable mammary carcinoma grown in female Wistar Furth rats; W/Fu, Wistar Furth; MtT, mammary tumor; MtTW10, a mammosomatotropic tumor obtained from Dr. U. Kim; MtT, mammosomatotropic tumor; MTW9-MtT, MTW9 supported by coinplantation of MTW10, MTW9-P, a variant of MTW9 obtained by perphenazine administration.

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food and water ad libitum. Under all these experimental conditions, animals appeared to have adequate food intake, as evidenced by continued weight gain.

Bilateral ovariectomies were performed under ether anesthesia, and surgical removal (resection) of MT was performed as previously described (10). Sham-ovariectomized rats were anesthetized with ether and the ovaries were exposed through a dorsal incision.

**Drug Administration.** Perphenazine (generously supplied by Dr. A. S. Watnick of the Schering Corp., Bloomfield, N. J.) was dissolved in 0.03 N HCl, diluted with 0.9% NaCl solution, and 4 mg/kg body weight were injected s.c. The drug was injected daily (between 11:00 a.m. and 1:00 p.m.) in a volume of 0.2 ml at a site distant from the tumor. 

Estradiol and steroid suspending vehicle (Armour Pharmaceutical Co., Kankakee, Ill.) were obtained from the Cancer Chemotherapy National Service, National Cancer Institute. Estradiol (10 μg/rat/day) was injected s.c. in 0.2 ml steroid suspending vehicle.

**Assay of Serum Prolactin.** Tail blood was collected between 2 and 4 p.m. (2 to 4 hr after the last perphenazine injection). Serum was removed after overnight clot retraction and stored at -70°C. Serum prolactin concentrations were determined by the double antibody radioimmunoassay with reagents supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases (NIAMD), NIH. Determinations were carried out with minor modifications of the standard procedure supplied with these reagents. Serum samples were analyzed in duplicate and, whenever possible, at 2 or more dilutions. The values obtained were averaged and the final concentrations were expressed as ng of NIAMD-Rat Prolactin-RP-1 per ml of serum. Aliquots of pooled sera from hypophysectomized female rats (Camm Research Institute, Wayne, N. J.) and from female rats bearing MT were routinely included to monitor variability between assays.

In our laboratory, serum prolactin values in intact cycling female rats vary between 9 and 110 ng/ml, with occasional levels of 156 to 240 ng/ml. High serum prolactin in occasional normal animals has also been reported by others (3).

**RESULTS**

The response of MTW9 to daily injections of perphenazine is illustrated in Chart 1. Rats implanted with MTW9 and given perphenazine, 4 mg/kg/day, develop palpable tumors between 26 and 40 days. These tumors usually attain a diameter ([length + width]/2) of 1.0 to 1.5 cm in 50 days. Tumor size is not expressed in volume because depth measurements are difficult to make. Chronic administration of perphenazine at the dose used produces serum prolactin concentrations of 150 to 600 ng/ml. In preliminary studies with long-term perphenazine-treated animals, we have observed that serum prolactin concentration returns to normal levels within 48 hr of perphenazine withdrawal. Chart 1 shows that, when perphenazine is withdrawn, serum prolactin decreases to normal but no tumor regression occurs. Ovariectomy of rats bearing MTW9-P causes striking regression of some tumors in 20 days and complete disappearance of others by 30 to 40 days (Chart 1). MTW9-P does not regress when perphenazine administration is stopped. Ten rats were maintained with MTW9-P for more than 3 months after perphenazine administration was withdrawn. None showed regression, about 80% were stationary, while the remainder showed slight growth. Chart 2 compares growth of MTW9-P in 3 representative animals withdrawn from perphenazine with those receiving the drug over a period of 136 days.

Ovariectomy results in tumor regression with or without cessation of perphenazine treatment. When daily perphenazine administration was continued (Chart 3), ovariectomy still led to tumor shrinkage. Removal of the ovaries in these perphenazine-maintained animals resulted in a decrease in serum prolactin. However, serum prolactin always remained well above normal. When perphenazine-maintained rats were subjected to sham ovariectomy, no tumor regression occurred, and serum prolactin remained elevated (data not shown). Ovariectomy resulted in at least a 50% reduction in average tumor diameter in all (over 20) MTW9-P-
Ovariectomy-responsive Rat Mammary Tumor

Regression of all tumors. MT resection in combination with ovariectomy further reduced serum prolactin levels to 34 ± 3.6 ng/ml.

DISCUSSION

MTW9-MtT does not regress after ovariectomy in spite of its demonstrated need for ovarian hormones. MacLeod et al. (9) showed that MTW9 would not grow in ovariectomized animals bearing MtT unless estradiol and progesterone were administered. Murota and Hollander (12) showed that ovariectomy lowered the serum prolactin of rats bearing bearing rats studied; 20% had complete regression of tumor by 50 days after surgery.

Chart 4 shows that daily administration of estradiol to rats with complete regression of tumor produced regrowth of MTW9-P within 30 to 40 days. Perphenazine readministration alone did not result in regrowth of tumor. Estradiol-induced regrowth of tumor also occurs without perphenazine administration (data not shown).

The behavior of MTW9-P (Chart 1) is strikingly different from that of MTW9-MtT. MTW9-MtT grows faster than MTW9-P and becomes palpable in 27 ± 1.0 days, attaining an average diameter of 1.0 to 3.0 cm in 36 ± 1.5 days. By the time mammary tumors are palpable, serum prolactin is much higher (500 to 7000 ng/ml) than in perphenazine-treated rats. MTW9-MtT-bearing rats do not live as long as perphenazine-treated animals. Rapid growth of MtT kills most animals by 50 to 60 days after tumor implantation. Resection of MtT prolongs the life of these animals; however, regrowth eventually kills them.

Chart 5 shows that resection of MtT in 4 rats bearing MTW9-MtT caused prompt regression of MtT. Chart 5 is representative of 40 MtT resections done in the course of this study; 28 (70%) showed a 20 to 80% reduction in MTW9 size and 6 (15%) completely regressed, while the remaining 6 (15%) remained stationary. Minimal tumor regression or absence of regression probably results from incomplete resection of the MtT.

Table 1 shows that removal of MtT causes serum prolactin to decrease 5- to 10-fold, to 102 ± 25 ng/ml. These reduced levels persist for 2 to 3 weeks until regrowth of MtT. Ovariectomy had no effect on the growth of MTW9-MtT (Chart 5), nor did it reduce the high serum prolactin concentration (Table 1). Ovariectomy has previously been shown to decrease serum prolactin in MtT-bearing rats (12). The reason for this difference is not clear but may be related to the different supporting tumor used in the present study. Ovariectomy had little effect on the rate of tumor regression during the first 7 days after MtT resection. However, 1 week after surgery, 3 out of 4 tumors in rats treated by MtT resection alone showed no further regression. In contrast, animals treated by both procedures showed complete regression of all tumors. MT resection in combination with ovariectomy further reduced serum prolactin levels to 34 ± 3.6 ng/ml.

**Table 1**

<table>
<thead>
<tr>
<th>Surgical procedure</th>
<th>Serum prolactin (ng/ml)</th>
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<tbody>
<tr>
<td>None (21)</td>
<td>3506 ± 411*</td>
</tr>
<tr>
<td>Ovariectomy (12)</td>
<td>3655 ± 642</td>
</tr>
<tr>
<td>MtT resection (15)</td>
<td>102 ± 25</td>
</tr>
<tr>
<td>MtT resection + ovariectomy (12)</td>
<td>34 ± 3.6</td>
</tr>
</tbody>
</table>

* Prolactin was measured at various intervals over a 3-week period after surgery.

† MtT's had average diameters of 2 to 4 cm.

‡ Numbers in parentheses, number of determinations.

§ Mean ± S.E.
MtT but to concentrations well above that required for growth of MTW9 when ovarian function was present. Chronic administration of perphenazine results in high serum prolactin levels which are, however, much lower than those achieved by growth of MtTW10. MTW9 transplants grow readily in perphenazine-treated rats, but the resultant tumor, although similar morphologically, is very different in behavior from the MTW9-MtT with respect to withdrawal of prolactin and estrogen. Resection of MtT causes a prompt fall in serum prolactin and MtT regression. Tumors regress completely when ovariectomy is combined with surgical removal of MtT. Cessation of perphenazine administration also causes a prompt fall in serum prolactin. Tumor growth is minimal, and no regression occurs in spite of normal serum prolactin concentration. However, MTW9-P regresses upon ovariectomy, whether perphenazine administration is continued or not.

The possibility that perphenazine altered MTW9-MtT cells in some permanent manner cannot be excluded. Animals bearing MTW9-MtT were treated with perphenazine in an attempt to show that the resulting tumor did not become ovariectomy dependent. Unfortunately, perphenazine could not be given to MTW9-MtT rats for more than 2 weeks, since the drug proved too toxic to such animals. Since MTW9-P remains ovariectomy responsive 10 days after the withdrawal of perphenazine, it is likely that MTW9-P represents a clone of MTW9 with a much lower requirement of prolactin. It is also likely that nearly all the tumor cells require ovarian hormones, since MTW9 will not grow at all in ovariectomized rats (9, 12). The partial regression of some MTW9-MtT tumors after MtT resection suggests that MTW9-MtT is heterogeneous. Perhaps the portion of the regression curve in Chart 5 that shows disappearance of tumor (between Days 9 and 16) when ovariectomy was carried out with resection represents a clone similar to MTW9-P. That MTW9 has the genetic information necessary to respond to ovariectomy has been reported by others (4). A transplantable variant of MTW9 which grows in intact rats has recently been shown to respond to ovariectomy (4). The failure of MTW9-MtT to regress after ovariectomy might represent inhibition of the response by some product of MtT. Growth hormone (5) or the elevated levels of insulin found in MtT-bearing rats (11) represent possibilities. The study of endocrine factors that inhibit response to ovariectomy of such tumors is of considerable interest.

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