

The Conversion of an Ovariectomy-nonresponsive to an Ovariectomy-responsive Mammary Tumor Strain¹

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SUMMARY

MTW9, a transplantable mammary tumor in Wistar Furth rats, shows little growth unless host serum prolactin is increased. This study compares the response to ovariectomy of MTW9-MtT, a tumor developed in rats bearing the mammosomatotropic tumor MtTW10 (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

MTW9³ 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 elevated serum prolactin is achieved by transplantation of pituitary glands beneath the renal capsule or by injection of a mixture of prolactin, growth hormone, estradiol, and progesterone (9).

Administration of perphenazine (4-[3-(2-chlorophenothiazin-10-yl)propyl]-1-piperazine ethanol) also results in elevated levels of serum prolactin (1). Pearson *et al.* (13) showed that chronic perphenazine administration in-

creased serum prolactin concentration and the growth rate of dimethylbenz(a)anthracene-induced carcinomas. Haloperidol (4-[4-*p*-chlorophenyl]-4-hydroxypiperidino]-4'-fluorobutyrophenone) and methyl dopa, 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 MtTW10 with rats given chronic perphenazine treatment. MTW9 obtained by daily injections of perphenazine regresses after ovariectomy. MTW9-MtT, the MT obtained by coimplantation of MtTW10, 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, and the MtT, with 2 volumes of Medium 199 (Microbiological Associates) containing 200 units penicillin and 200 µg streptomycin per ml. One or more MTW9 tumors were used for each transplant generation. MtT suspension (0.2 ml) was injected s.c. into the lower scapular region and the MT suspension (0.6 ml) was injected into the right flank with a 13-gauge needle. Both tumors (MtTW10 and MTW9) were injected into approximately one-half of the recipients in an experimental group, while the other one-half received only MTW9 (from the same tumor source), plus daily s.c. injections of perphenazine (4.0 mg/kg). Administration of perphenazine deranges body temperature control (6) and tends to weaken the animals. Perphenazine-treated rats and controls for this study were housed at 27-28° with ready access to food. MTW9-MtT-bearing rats were maintained at 22-23°. Some MTW9-MtT rats were also maintained at 27-28°. This elevated room temperature did not affect the growth rate of MTW9-MtT. Animals were weighed weekly and tumor size was measured 3 times weekly. All animals were housed 5 to a cage in rooms with 12-hr light and dark cycles and were allowed

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³ The abbreviations used are: MTW9, a transplantable mammary carcinoma grown in female Wistar Furth rats; W/Fu, Wistar Furth; MT, mammary tumor; MtTW10, a mammosomatotropic tumor obtained from Dr. U. Kim; MtT, mammosomatotropic tumor; MTW9-MtT, MTW9 supported by coimplantation of MtTW10; 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 MtT 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. 17β -Estradiol and steroid suspending vehicle (Armour Pharmaceutical Co., Kankakee, Ill.) were obtained from the Cancer Chemotherapy National Service, National Cancer Institute. Estradiol (10 μ g/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° . 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 MtT 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 disappear-

ance 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-

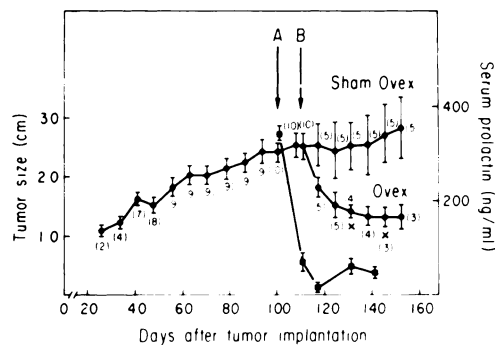


Chart 1. Growth of MTW9-P. MTW9 was implanted 24 hr prior to the start of perphenazine injections at Day 0. Perphenazine (4 mg/kg/day) was given for 100 days and stopped at Point A. At Point B, one-half ($n = 5$) of the animals were ovariectomized (Ovex) and the other half were sham-ovariectomized (Sham Ovex). Tumor size (●) is expressed as average diameter [(length + width)/2]. Small changes in average diameter represent substantial changes in tumor volume. Completely regressed tumors were not entered into average size. Serum prolactin concentrations (■) did not differ significantly between groups (Ovex versus Sham Ovex), and the values are pooled. Numbers in parentheses, number of animals with tumor at each point; x, points at which complete regression of tumor occurred; values are expressed as mean \pm S.E. By Day 144, 2 out of 5 tumors in the Ovex group were no longer palpable.

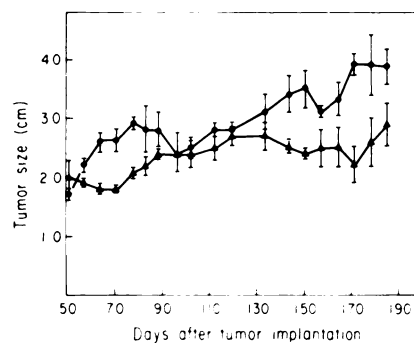


Chart 2. Effect of perphenazine withdrawal on growth of MTW9-P. MTW9 was implanted into 6 rats 24 hr prior to start of perphenazine injections (4 mg/kg/day). Fifty days after tumor implantation, perphenazine was withdrawn from 3 animals (Δ) and continued for the other 3 (\bullet). Tumors were measured in both groups for another 136 days. Tumor size is expressed as average diameter [(length + width)/2] (mean \pm S.E.).

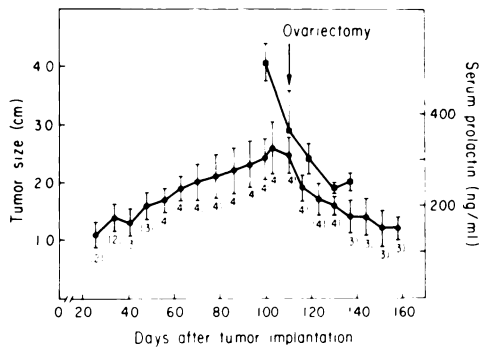


Chart 3. Effect of ovariectomy on growth of MTW9-P in rats receiving daily injections of perphenazine. MTW9 was implanted 24 hr prior to start of perphenazine injections at Day 0. All animals were ovariectomized on Day 110. Tumor size (●) and serum prolactin (■) were measured at intervals noted. Numbers in parentheses, number of animals. By Day 137 (27 days after surgery), 1 animal no longer had palpable tumor. Tumor size is expressed as average diameter [(length + width)/2]. All values are expressed as mean ± S.E.

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).

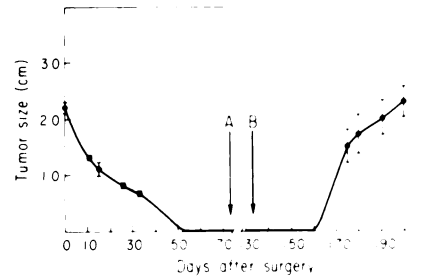


Chart 4. Effect of estradiol administration on regrowth of MTW9-P. Perphenazine injections were stopped and 3 animals were ovariectomized at Day 0. Perphenazine injections were started again at Point A. Estradiol (10 µg/day) administration was started at Point B. Results are expressed as average tumor diameter [(length + width)/2] (mean ± S.E.).

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.

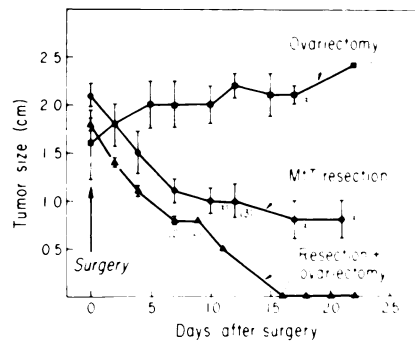


Chart 5. Effect of resection, ovariectomy, and resection plus ovariectomy on growth of MTW9-MtT. Rats bearing MTW9-MtT were subjected to MtT resection (●), ovariectomy (■), or ovariectomy + MtT resection (▲) on Day 0. Four animals were subjected to each surgical procedure. Tumors were measured at intervals noted. Numbers in parentheses, number of animals remaining with palpable tumor. In the ovariectomized group, 1 animal had died by Day 17 and 3 died by Day 24. Tumor size is expressed as average diameter [(length + width)/2] (mean ± S.E.).

Chart 5 shows that resection of MtT in 4 rats bearing MTW9-MtT caused prompt regression of MT. 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 1st 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. MtT 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

Table 1

Effect of resection, ovariectomy, and ovariectomy plus resection on serum prolactin concentration^a in MtT-^b plus MT-bearing rats

Surgical procedure	Serum prolactin (ng/ml)
None (21) ^c	3506 ± 411 ^d
Ovariectomy (12)	3655 ± 642
MtT resection (15)	102 ± 25
MtT resection + ovariectomy (12)	34 ± 3.6

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

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

^c Numbers in parentheses, number of determinations.

^d 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 MT 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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