Effect of High-Dose Progesterone on Growth of Rat Mammary Carcinoma

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ABSTRACT

Growth of the transplantable rat mammary carcinoma, MTW9, is stimulated by elevated serum prolactin concentrations. When serum prolactin is increased by coimplantation with a mammosomatotropic tumor, the mammary tumor, MTW9-MtT, does not regress after ovariectomy, but rapid growth ceases. In contrast, when MTW9 is grown in rats with high serum prolactin produced by p.o. administration of perphenazine, the tumor, MTW9-P, does regress after ovariectomy. When perphenazine treatment is discontinued, the tumor, now referred to as MTW9-PD, stops growing but still regresses after ovariectomy.

The present report examines the effects of progesterone on growth of MTW9 mammary tumors in ovariectomized rats. Administration of progesterone (10 mg/day) to rats bearing MTW9-PD completely prevented ovariectomy-induced regression but did not stimulate tumor growth or increase serum prolactin. Cessation of progesterone administration caused tumor regression. Administration of neither estrogens nor 17-hydroxyprogesterone caproate to rats bearing MTW9-PD failed to affect growth.

When estradiol benzoate (5 μg/day) was combined with progesterone, the ability of progesterone to stimulate growth of MTW9-MtT after ovariectomy was lost. Progesterone (10 mg/day) also retarded the rapid and complete regression of MTW9-MtT which occurs when ovariectomy is combined with surgical removal of the mammosomatotropic tumor. No effects of progesterone were observed on growth of either MTW9-MtT or MTW9-PD in intact rats. Progesterone may inhibit mammary tumor regression and stimulate tumor growth after ovariectomy by acting directly on tumor cells or indirectly by stimulating the secretion of a pituitary growth factor.

INTRODUCTION

MTW9* is a carcinogen-induced rat mammary carcinoma which requires elevated levels of serum prolactin and physiological concentrations of ovarian steroids for growth (12, 13, 16). High serum prolactin concentrations can be achieved by coimplantation with a MtT or by daily administration of the tranquilizing drug, perphenazine (4-[3-(2-chlorophenothiazin-10-yl)propyl]-1-piperazineethanol) (6).

MTW9-MtT is grown by coimplantation of MTW9 with MtTW10, a MtT which secretes prolactin, growth hormone, and probably ACTH (5). MTW9-MtT stops growing after ovariectomy but does not regress; regression occurs after surgical removal of the MtT when serum prolactin falls to normal. MTW9-P is grown by administration of perphenazine to rats inoculated with MTW9 and, in contrast to MTW9-MtT, regresses after ovariectomy whether serum prolactin is normal or high (6). MTW9-MtT and MTW9-P are identical by light microscopy but differ in prolactin and estradiol receptor content (4, 19).

Early observations concerning growth of MTW9-MtT in ovariectomized rats showed that ovarian hormones were required for growth. Ovariectomy at the time of tumor implantation or shortly thereafter prevented mammary tumor growth. Daily administration of estradiol, progesterone, or a combination of both steroids that was started at the time of tumor implantation allowed mammary tumor growth to occur. Greater tumor growth occurred in ovariectomized rats receiving only progesterone from time of tumor implantation than in rats receiving only estradiol and indicated that progesterone, under the appropriate experimental conditions, can stimulate growth of MTW9-MtT (15).

These studies, the reports of Huggins et al., and other studies, showing stimulation (8–11) and inhibition (14, 20, 23) of carcinogen-induced mammary tumor growth by progesterone, led us to reexamine the influence of progesterone in the growth of MTW9 mammary tumors.

We recently reported that implantation of MtTW10 into rats bearing MTW9-PD completely prevents ovariectomy-induced tumor regression. Attempts to simulate the endocrine environment of MtT-bearing rats by administration of prolactin, growth hormone, or 17-hydroxyprogesterone caproate to rats bearing MTW9-PD failed to prevent ovariectomy-induced regression and led us to suggest that MtTW10 secretes a substance other than prolactin which inhibits ovariectomy-induced regression (5).

We now report that progesterone affects the growth of MTW9 mammary tumors in several ways that differ from the early studies of MacLeod et al. (15) using MTW9 and from those of Huggins et al. and others using carcinogen-induced mammary tumors (1, 8–11). It will be shown that progesterone prevents ovariectomy-induced regression of MTW9-PD and also permits continued growth of MTW9-MtT in ovariectomized rats. Progesterone is the only hormone tested thus far that prevents ovariectomy-induced regression of MTW9-PD.

MATERIALS AND METHODS

Animals, Tumors, and Related Procedures. MTW9 and MtTW10 were obtained from Dr. U. Kim, Roswell Park Memorial...
Institute, Buffalo, N. Y., and from Dr. Pietro Gullino of the National Cancer Institute, NIH, Bethesda, Md. The source of Wistar/Furth rats, inoculation of MTW10, and the maintenance of tumor-bearing rats have been described previously (6). MTW9 was implanted as a fine mince (0.2 ml/rat) into the right inguinal region using a 1.0-ml tuberculin syringe and a 13-gauge needle. For the present studies, MTW9-P was grown in rats implanted with MTW9 by p.o. administration of perphenazine instead of by daily injection as described previously (6). Concentrated perphenazine (Trilafon), generously supplied by Schering Corp., Kenilworth, N. J., was diluted and administered in drinking water at a perphenazine concentration of 5 mg/100 ml and at an estimated average dosage of 0.4 to 0.5 mg/day. MTW9-P obtained in this way has a longer latency period than described previously and does not grow much larger than approximately 1.5 cm in average diameter (3 diameters/3); however, it has the same biological properties as described previously (6). MTW9-PD was obtained by administration of perphenazine followed by withdrawal from the drug when implants of MTW9 had attained a minimum average diameter of 1.0 cm. Most MTW9-PD mammary tumors do not grow; the hosts have normal concentrations of serum prolactin and show mammary tumor regression after ovariectomy (6). Procedures for ovariectomies and surgical removal of MTW10 have been described (6, 15).

**Administration of Progestins and Estrogens.** Progesterone was obtained from Steraloids Inc., Wilton, N. H., and the long-acting synthetic steroids, 17-hydroxyprogesterone caproate and estradiol valerate (Delestrogen) were generously supplied by E. R. Squibb and Sons, Princeton, N. J. Estradiol benzoate was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Suspensions of 1, 4, 5, and 10 mg progesterone per ml and 4 and 10 mg per ml of 17-hydroxyprogesterone caproate were prepared in steroid-suspending vehicle (0.9% NaCl solution, 0.5% carboxymethylcellulose, 0.4% polyoxyethylene sorbitan monooleate, and 0.9% benzyl alcohol in water). The total daily doses of progesterone (1, 4, 5, or 10 mg) or of 17-hydroxyprogesterone caproate (4 or 10 mg) were administered as 2 s.c. injections (at 9 a.m. and 4 p.m.) made at sites distant from the tumors. Daily s.c. injections of estradiol benzoate (5 or 10 μg/rat) were administered in steroid-suspending vehicle. Progesterone, 17-hydroxyprogesterone caproate, and estradiol benzoate were administered 5 times weekly. Estradiol valerate (10 mg/ml), a slow release estrogen, was diluted with hydroxyprogesterone caproate at 4 and 10 mg/day did not prevent ovariectomy-induced regression of MTW9-P and had no significant effect on either the extent or rate of tumor regression.

In contrast to the complete inhibition of ovariectomy-induced regression in progesterone-treated rats, tumor regression occurred in estrogen-treated rats. Chart 3 shows that neither 10 μg of estradiol benzoate per day nor 40 μg of estradiol valerate per week were able to significantly retard tumor regression. A similar treatment regimen using estradiol valerate has been shown to restore growth of DMBA-induced mammary tumors after ovariectomy-induced regression (3). Chart 3 shows that tumor regression also occurred in rats receiving 15 μg per week of estradiol valerate but that regression was not as extensive as that observed with other estrogen treatment regimens. This treatment regimen showed a statistically significant difference (p < 0.001) when compared with vehicle-injected rats.

**Assay of Serum Prolactin.** Blood for analyses was collected from rats bled via the tail vein by negative pressure (18) or from the trunk after decapitation. Serum was removed after overnight clot retraction and stored at -20° until assayed. Serum prolactin concentrations were determined as described previously (6) by double antibody radioimmunoassay using reagents supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases, NIH.

**RESULTS**

**Effect of Progesterone on Ovariectomy-induced Regression of MTW9-PD.** Chart 1 shows that daily administration of progesterone (10 mg/day) for 10 days to rats bearing MTW9-PD had little effect on cessation of tumor growth normally observed after withdrawal of perphenazine (6). Ovariectomy of control animals led to regression of the mammary tumors. In contrast, progesterone-treated rats failed to show ovariectomy-induced regression. After 50 days of progesterone administration, the hormone was withdrawn, and tumors regressed to less than 80% of their original diameters. Daily administration of 5 mg progesterone, but not of 1 mg progesterone, also prevented ovariectomy-induced regression of MTW9-PD in a manner similar to that with 10 mg per day (data not shown).

Ovariectomized rats bearing MTW9-PD and treated with progesterone (10 mg/day) had serum prolactin concentrations of 13 ± 4 (S.E.) ng/ml (n = 11), while vehicle-treated ovariectomized control rats had 17 ± 6 ng/ml (n = 11), showing that progesterone treatment produced little change in serum prolactin. Hence, inhibition of ovariectomy-induced regression elicited by high-dose progesterone is not due to an increase in serum prolactin.

Chart 2 confirms and extends our previous observations that large doses of 17-hydroxyprogesterone caproate fail to prevent ovariectomy-induced regression of MTW9-PD (5). Treatment with hydroxyprogesterone caproate at 4 and 10 mg/day did not prevent ovariectomy-induced regression of MTW9-PD and had no significant effect on either the extent or rate of tumor regression.
Tumors were 1.0 to 2.0 cm in average diameter before start of treatment. Points, mean values; bars, S.E. Change in average tumor diameter (3 diameters/3) is expressed as a percentage of the average diameter at start of treatment (initial tumor diameter). Mammary tumors measured approximately 1.0 cm on day of ovariectomy. Rats given injections of either estradiol benzoate (10 μg/day in steroid-suspending vehicle; n = 2) or estradiol valerate [15 μg/week in sesame oil (n = 6) or 40 μg/week in sesame oil (n = 4)]. Control rats (n = 6) received injections of vehicle. Change in average tumor diameter (3 diameters/3) is expressed as a percentage of the average diameter at the day of ovariectomy (initial tumor diameter). Mammary tumors measured 1.0 cm on day of ovariectomy. Points, mean values; bars, S.E. Number of animals is the same for all animals regardless of the vehicle injected.

Effect of Ovariectomy on Growth of MTW9-PD in Rats Receiving Daily Injections of Hydroxyprogesterone Caproate. Rats bearing MTW9-PD received 2 daily s.c. injections of hydroxyprogesterone caproate (HPC), 10 mg/day (n = 6) or 4 mg/day (n = 3) or equal volumes of steroid-suspending vehicle (controls; n = 4). After treatment for 12 days, all rats were ovariectomized, and hydroxyprogesterone caproate treatment continued until termination of the experiment. Change in average tumor diameter (3 diameters/3) is expressed as a percentage of the average diameter at the start of treatment (initial tumor diameter). Mammary tumors were 1.0 to 2.0 cm in average diameter before start of treatment. Points, mean values; bars, S.E.

Effect of Progesterone on Growth of MTW9-MtT in Ovariectomized Rats. MTW9-MtT stops growing after removal of the ovaries; hence, it seemed logical to determine whether high-dose progesterone would also prevent this response to ovariectomy. Chart 4 shows that administration of 4 mg progesterone per day reversed the previously described growth arrest of MTW9-MtT which occurs after ovariectomy (6). This study was carried out on tumors measuring 1 to 2 cm in diameter which grow rapidly, as shown by the growth of tumors in intact vehicle-injected control rats. The progesterone-stimulated growth rate in ovariectomized rats was similar in these controls.

In spite of the large doses of progesterone required to inhibit ovariectomy-induced regression of MTW9-PD (5 and 10 mg/day) and those used to stimulate growth of MTW9-MtT after ovariectomy (4 mg/day), no effects of progesterone were observed on the growth of MTW9-P, MTW9-PD, or MTW9-MtT in intact rats (data not shown). Chart 5 shows the effect of estrogen treatment with or without progesterone on the growth of MTW9-MtT after ovariectomy. The rapid growth of MTW9-MtT in intact hosts is illustrated by the control line. In sharp contrast to the ability of progesterone to stimulate tumor growth after ovariectomy (Chart 4), 3 different estrogen treatment regimens, 5 μg estradiol benzoate per day and 20 and 40 μg estradiol valerate per week, failed to significantly affect growth of MTW9-MtT after ovariectomy. The change in tumor size after ovariectomy was similar for all animals regardless of the vehicle injected. The rapid growth of MTW9-MtT in intact hosts is illustrated by the control line. In sharp contrast to the ability of progesterone to stimulate tumor growth after ovariectomy, the addition of estradiol benzoate (5 μg/day) to the progesterone treatment regimen completely eliminated the growth-stimulatory effect of progesterone.

Surgical removal of MTW10 from hosts bearing MTW9-MtT controls; however, tumor growth was not stimulated and regression occurred.

Table 1 summarizes the effects of estrogen and progesterone treatment on growth of MTW9-MtT after ovariectomy (Charts 4 and 5) and evaluates the data 13 to 15 days after ovariectomy. Analysis of variance showed highly significant differences between treatment groups. Progesterone stimulated tumor growth which was not significantly different from that observed in intact controls. Estrogen treatment alone and in combination with progesterone showed some growth stimulation which was not significantly different from ovariectomized control rats but was significantly different from growth seen with progesterone alone (p < 0.025).

Surgical removal of MTW10 from hosts bearing MTW9-MtT...
results in regression of the mammary tumor. We have shown that when surgical removal of MIT and ovariectomy are combined, regression of MTW9-MIT is more rapid and extensive than after removal of the MIT alone (6). Chart 6 shows that progesterone administration prevented a large portion of the mammary tumor regression caused by combined MIT removal and ovariectomy, although the initial rate of tumor regression was similar. Statistically significant differences between progesterone-treated rats and controls were observed from Day 13 ($p < 0.01$) to the end of the experiment ($p < 0.001$).

**DISCUSSION**

The present study is concerned with effects of progesterone on mammary tumor growth and on inhibition of mammary tumor regression, effects which are manifest only in ovariectomized rats. Our findings differ from those of Huggins et al. and others, who showed that progesterone stimulated the rate of carcinogen-induced mammary tumor growth in intact rats (1, 8-11). In our studies, the administration of progesterone to ovariectomized tumor-bearing hosts restored growth of MTW9-MIT and prevented ovariectomy-induced regression of MTW9-PD. The same treatment does not stimulate tumor growth in intact animals bearing either mammary tumor. This is in contrast to the work of Huggins et al. (9, 10), who treated intact rats with progesterone shortly after dimethylbenzanthracene administration. Progesterone was shown to decrease tumor latency period, increase the number of tumors, and increase tumor growth rate. The present study is concerned with a different experimental mammary tumor system, i.e., tumors transplantable in syngeneic rats which have predictable biological behavior. One tumor (MTW9-P) regresses after ovariectomy and another tumor (MTW9-MIT) does not (6). Our study demonstrates an effect of progesterone on both of these tumors, but only in ovariectomized and not in intact rats.

Macleod et al. (15) showed that MTW9, when coimplanted with MIT, failed to grow in ovariectomized rats. Estradiol or progesterone treatment elicited some growth, but only administration of both steroids allowed the transplantable mammary tumor to grow at the same rate as did intact hosts. The present study does not involve the initiation and early growth phase of tumor implants but deals with fully developed tumors.

MTW9-MIT stops growing but does not regress after ovariectomy, thus maintaining its size (Chart 4); administration of estrogen does not stimulate tumor growth. This lack of an estrogen effect is in contrast to the growth-stimulatory effect reported by Macleod et al. (15). However, Macleod et al. studied initiation and early growth of MTW9, whereas the present study is concerned with the fully developed mammary tumor. Hence, estrogen appears to stimulate growth of MTW9 only during its early growth phase. Administration of progesterone permits growth of the mammary tumor in ovariectomized rats at almost the same rate as in intact rats (Chart 4). This
also contrasts with the findings of Macleod et al. (15) who showed that progesterone alone did not allow growth of tumor implants similar to that observed in intact animals. The stimulatory effect of progesterone on growth of MTW9-MtT in ovariectomized rats was completely reversed by the addition of estrogen to the progesterone treatment regimen (Chart 5). This is similar to the growth-inhibitory effect of the same steroid combination on dimethylbenzanthracene-induced mammary tumors observed by Huggins and Yang (10) but differs from the stimulatory effect of the 2 steroids on growth of MTW9 implants as reported by Macleod et al. (15).

Serum progesterone concentrations are about 2- to 3-fold higher than normal in rats bearing either MTW9-MtT or MTW9-P (7). These increases are probably due to the luteotrop effect of prolactin (10). The lack of any growth response to progesterone in intact rats bearing MTW9 tumors may be related to elevated serum progesterone. The high serum progesterone may be sufficient to produce the maximum physiological effect(s) and any additional hormone would then be ineffective in stimulating tumor growth. Ovariectomy leads to very low concentrations of progesterone in tumor-bearing rats. The progesterone effects described in the present study occurred only after ovariectomy, i.e., at a time when serum progesterone was decreased to very low levels. Hence, the effect of steroid administration may have been due to replacement of the progesterone loss. This interpretation would explain the effects of progesterone on restoration of MTW9-MtT growth after ovariectomy as well as on prevention of ovariectomy-induced regression of MTW9-PD.

We have suggested that the failure of MTW9-MtT to regress after ovariectomy and the ovariectomy-induced regression elicited by surgical removal of the MT implied that the MT in some way prevented ovariectomy-induced regression. We were unable to simulate the inhibitory effect of an MT transplant by administration of the known secretory products of MtTW10 (prolactin, growth hormone, or ACTH). Only implantation of MtTW10 prevented ovariectomy-induced regression of MTW9-PD. Either MTW10 secretes a factor which stimulates growth of MTW9 in the absence of the ovaries or a combination of hormones present in MT-bearing rats prevents ovariectomy-induced regression (5). Progesterone prevents ovariectomy-induced regression of MTW-PD and in this respect simulates MtTW10, which also prevents ovariectomy-induced regression of MTW9-PD (5). However, it is unlikely that progesterone is secreted by a transplantable pituitary tumor or that it is responsible for lack of ovariectomy-induced regression in MT-bearing rats. Serum progesterone concentrations are essentially the same and only moderately elevated (2 to 3 times normal) in animals bearing MTW9-MtT and MTW9-P (7); however, in both cases, serum progesterone is very low after ovariectomy, yet MTW9-PD regresses and MTW9-MtT does not. High serum progesterone may act indirectly by stimulating pituitary secretion of a substance (not prolactin, growth hormone, or ACTH) which inhibits ovariectomy-induced mammary tumor regression or progesterone may inhibit by a direct action on tumor cells.

The specificity of the progesterone effect on MTW9-PD tumor inhibition and the lack of any growth effect with 17-hydroxyprogesterone caproate is of interest. The long-acting prostaglandinal agent, 17-hydroxyprogesterone caproate, was completely unable to prevent ovariectomy-induced regression of MTW9-PD. However, some of the biological activities described for this compound are quite different from those of progesterone (21). In terms of tumor growth needs, hydroxy-progesterone caproate may not be as biologically active as progesterone or it may be metabolized differently than is the physiological hormone.

MTW9-MtT and MTW9-PD grow in very different endocrine environments, which may influence the way hormones are metabolized. MTW9-MtT grows in a highly abnormal environment, whereas animals bearing MTW9-PD probably have normal concentrations of most hormones (7). These differences may influence the way progesterone is metabolized. The pattern of progesterone metabolites has been shown to vary with the endocrine status of the animal (2). The hormonal environment can also influence the way a mammary tumor metabolizes steroids (17). Progesterone can influence synthesis of its own receptor (22). Hence, it is possible that in MTW9-PD-bearing rats progesterone may be metabolized to a more active growth factor. It would be of interest to compare the metabolic products of progesterone in animals bearing MTW9-MtT, MTW9-P, or MTW9-PD and those in normal rats.

We have shown that progesterone plays an important role in growth and regression of MTW9. Whether the actions of progesterone on MTW9 tumors are related to physiologically important processes or are pharmacological effects remains to be clarified.

Although the mechanism(s) by which progesterone influences growth and regression in the MTW9 mammary tumor system is not understood, it is clear, as reported by others, that progesterone is an important growth factor for mammary cancer (18).

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