Eur J Cancer Clin Oncol, Vol. 18, No. 5, pp. 495–505, 1982.
Printed in Great Britain.

0277-5379/82/050495-10$03.00/0

Enhanced and Reversed Growth *In Vivo* of a Pregnancy-dependent Mouse Mammary Tumor (TPDMT-4) by a Gonadotropin-releasing Hormone Agonist Analog*

AKIO MATSUZAWA† and TADASHI YAMAMOTO‡

†*Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, P. O. Takanawa, Tokyo* 108 *and* ‡*Tokyo Metropolitan Institute of Medical Science, Honkomagome, Bunkyo-ku, Tokyo* 113, *Japan*

Abstract—*TPDMT-4 pregnancy-dependent mammary tumors grow continuously in DDD female mice carrying pituitary isografts (PI) ectopically or bearing an s.c. 17β-estradiol plus progesterone (EP) pellet. A gonadotropin-releasing hormone (GnRH) agonist, (D-leucyl^6, des-glycyl-NH$_2^{10}$, prolyl-ethylamide^9) GnRH (TAP-144), was examined for its antitumor activity in these experimental systems. TAP-144 was injected i.p. at doses of* 300 *and* 600 μg 6 *times weekly, when tumors grew to significant sizes. TAP-144 enhanced tumor growth during the first* 2 *weeks and subsequently reversed it in a dose-related manner in PI-bearing mice. The agonist did not affect tumor growth in the absence of ovaries in these mice. In ovariectomized mice, TAP-144 enhanced EP pellet-induced tumor growth but never reversed it. In ovariectomized, PI-bearing mice, TAP-144 first enhanced and subsequently reversed tumor regrowth induced by ovarian grafts to a greater extent when commencing it simultaneously with ovarian grafting than* 30 *days before it. The agonist also exerted the dual effects on TPDMT-4V ovarian-dependent subline tumors in the absence of PI. In TAP-144-treated mice, enhanced tumor growth was related to many solid corpora lutea in ovaries and fully developed mammary glands, but reversed growth was related to atrophied luteal components and mammary glands. The results suggest that TAP-144 enhances tumor growth first via its stimulative action on the pituitary–ovarian axis and causes tumor regression later via its direct inhibitory action on ovaries.*

INTRODUCTION

THE DECAPEPTIDE gonadotropin-releasing hormone (GnRH) agonist, specifically (D-leucyl^6, des-glycyl-NH$_2^{10}$, prolyl-ethylamide^9) GnRH (TAP-144), is 50–80 times more potent in causing ovulation in the diestrus rat [1] and 3–5 times more active in stimulating the release of LH and FSH from rat pituitaries *in vitro* [2] as compared with the natural hormone. Chronic administration of large doses of TAP-144 gave rise to the cessation of cycling and atrophy of the ovary and the uterus in the mature animal [3]. In addition, another agonist inhibits FSH-induced increase of estrogen (E) and progesterone (P) production by rat ovarian granulosa cells *in vitro* [4, 5]. As expected from such effects mimicking ovariectomy, TAP-144 has elicited regression of ovarian-dependent rat mammary tumors developing spontaneously [6] or induced by 7,12-dimethylbenz(a)anthracene (DMBA) [7–9].

A very stable, transplantable, pregnancy-dependent mammory tumor line, TPDMT-4, was successfully isolated in DDD mice [10]. TPDMT-4 tumors are characterized by pregnancy-dependent growth. They produce practically no growth in intact virgin mice, but continued growth in virgins with ectopic pituitary isografts (PI) [11]. The PI free from hypothalamic control produces a pseudopregnant state by producing primarily prolactin with luteotropic effect on the ovary and mammotropic effect on the mammary gland [12]. Both E and P from the ovary are essential for the tumor growth in the pseudopregnant hosts.

Accepted 29 December 1981.
*This work was supported in part by a grant-in-aid for cancer research from the Ministry of Education, Science, and Culture, Japan.

Continuous tumor growth also occurs in virgins with an s.c. 17β-estradiol and P (EP) pellet [11]. In these hosts tumors cannot grow in the absence of pituitary hormones [13]. Demonstration of E and P receptors, and E control of P receptor synthesis has supported the importance of both steroids for the tumor growth [14, 15].

These experimental systems have provided unique models for studies on endrocrine therapy of hormone-dependent breast cancer. A steroidal antiestrogen, epithiostanol, a nonsteroidal antiestrogen, tamoxifen, and a steroidal androgen, testosterone, were comparable to ovariectomy in causing the regression of TPDMT-4 tumors in PI-bearing mice [16, 17]. These compounds are similar in eliciting atrophy of the ovary. Thus, we investigated the effect of TAP-144 on this particular model system.

MATERIALS AND METHODS

Mice

DDD mice were bred and maintained in the mouse colony operated by the Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo. The origin and the characteristics of the strain of mice have been described elsewhere [18]. Six to 7-week-old virgin mice served as recipients, 2 to 4-month-old male and female mice as donors of PI, and 50-day-old females as donors of ovaries. All mice were fed Laboratory Chow F-2 (Funabashi Nojo Co., Ltd., Funabashi-city, Japan), given water *ad libitum* and housed 4–8 per cage.

Tumors

TPDMT-4 tumors at transplant generations 8–10 and TPDMT-4V tumors at generation 4 were used. The properties of the TPDMT-4 tumors have been described in detail [10, 11]. TPDMT-4V is an ovarian-dependent subline which was established from a sporadic outgrowth of a TPDMT-4 tumor at generation 29 in a virgin host [19]. The new line is characterized by slow but progressive growth in virgins and by insignificant growth in ovariectomized mice. Both E and P are important for the tumor growth. Mice implanted with tumors were inspected by palpation and measured for the 2 perpendicular diameters of a palpable tumor with calipers twice weekly. The arithmetic mean of these 2 diameters was designated as the tumor diameter, which was used to express tumor growth.

Treatments

In Experiments 1 and 2 a TPDMT-4 tumor from a late-pregnant host was cut into approximately 2 × 2 × 2-mm pieces. A tumor piece and 3 PI were implanted together into the right inguinal fat pad of each recipient. When tumors grew to 3–8 and 15.5–21 mm in diameter on days 27 and 74 of implantation in Experiments 1 and 2 respectively, mice were divided into groups, each containing tumors of similar diameters and latency period. In Experiment 3, TPDMT-4 tumors were grown in mice ovariectomized and given an s.c. implant of an EP pellet containing 0.16 mg 17β-estradiol, 39.90 mg P and 9.94 mg cholesterol into the back. When tumors reached 4–10 mm in diameter 17 days after implantation, mice were divided into 3 groups similar in tumor diameters and latency periods. In Experiment 4 an approximately 2 × 2 × 2-mm TPDMT-4V tumor piece was implanted into the right inguinal fat pad of each recipient. When tumors grew to 4–14 mm in diameter 55 days after implantation, mice were divided into 3 groups in a similar way. Each group of mice in Experiments 1–4 was treated as described in Table 1: the indicated dose of TAP-144 was dissolved in 0.1 ml saline solution and injected i.p. 6 times weekly for the indicated period, and intact and ovariectomized controls received 0.1 ml saline solution alone. In Experiment 5, TPDMT-4 tumors were implanted together with 3 PI in the same manner. When they grew to 9–17.5 mm in diameter on day 58 of implantation, all mice were ovariectomized and divided into 3 groups, each containing 7 tumors with similar diameters and latency periods. They all received an implant of 2 ovarian grafts from a syngeneic mouse into the left inguinal fat pad on day 30 of ovariectomy. The first group of mice were injected with saline solution alone, the second with saline solution before ovarian grafting and with 600 μg TAP-144 after that, and the last with 600 μg TAP-144 6 times per week for 101 days.

Morphology

At the termination of treatment all mice were killed, and the uteri, ovaries, adrenal glands and tumors were dissected, cleaned of adhering fat, and weighed wet. Tumors and ovaries were fixed in 10% formalin solution, processed routinely, and stained with hematoxylin and eosin for histologic study. The thoracic mammary glands were examined as whole mounts in Experiments 1 and 2 and the degree of their development was classified into 5 grades according to criteria described in [17, 19].

Table 1. Tumor diameter and weight and organ weights in control and TAP-144-treated groups in Experiments 1–4

Experiment* (duration of treatment)	Treatment (No. of mice treated)	Tumor diameter (mm) Initial	Final	Tumor weight (g)	Ovarian weight (mg)	Uterine weight (mg)	Adrenal weight (mg)
1 (54 days)	Saline solution (11)	5.3 ± 0.5†	15.0 ± 1.1	1.43 ± 0.30	20.0 ± 0.6	108 ± 3	9.2 ± 0.3
	TAP-144, 600 μg (11)	5.3 ± 0.5	4.9 ± 1.0‡	0.15 ± 0.05‡	12.4 ± 0.6‡	115 ± 9	9.4 ± 0.3
	TAP-144, 300 μg (11)	5.1 ± 0.4	6.2 ± 1.0‡	0.28 ± 0.12‡	12.7 ± 0.4‡	159 ± 11‡	9.4 ± 0.2
	Ovariectomy (11)	5.2 ± 0.4	1.9 ± 0.4‡	0.022 ± 0.004‡		32 ± 6‡	9.3 ± 0.3
2 (44 days)	Ovariectomy (6)	18.5 ± 1.0	10.2 ± 0.8	0.43 ± 0.08		29 ± 1	
	Ovariectomy + TAP-144, 600 μg (6)	18.7 ± 1.0	10.8 ± 0.8	0.48 ± 0.10		55 ± 4‡	
3 (44 days)	Saline solution (7)	5.8 ± 0.6	9.7 ± 1.7	0.55 ± 0.21			
	TAP-144, 600 μg (7)	5.7 ± 0.9	16.1 ± 2.3§	2.41 ± 0.82§			
	TAP-144, 300 μg (7)	5.9 ± 0.8	11.7 ± 2.7	1.23 ± 0.67			
4 (50 days)	Saline solution (12)	8.1 ± 0.9	13.2 ± 1.8	1.17 ± 0.36	16.0 ± 0.2	189 ± 20	8.5 ± 0.2
	TAP-144, 600 μg (12)	8.1 ± 0.8	9.6 ± 1.1	0.40 ± 0.08§	12.5 ± 0.5‡	160 ± 9	9.0 ± 0.2
	Ovariectomy (6)	9.1 ± 2.1	4.4 ± 1.6	0.10 ± 0.05		28 ± 1	8.5 ± 0.3

*TPDMT-4 tumors were grown in PI-bearing mice in Experiments 1 and 2 and in EP pellet-bearing, ovariectomized mice in Experiment 3, and TPDMT-4V tumors in virgin mice in Experiment 4. When tumors reached the initial diameters, ovariectomy was made and treatments were started.

†Mean ± S.E.

‡Significantly different from the control value at $P < 0.01$.

§Significantly different from the control value at $P < 0.05$.

Statistics

Student's *t*-test was used for statistical analysis. The difference was evaluated as significant at $P < 0.05$.

RESULTS

Effect of TAP-144 on growth of TPDMT-4 tumors in PI-bearing mice

In accord with the previous results [16, 17], tumors of control PI-bearing mice showed continued growth and those of ovariectomized mice progressive regression throughout the observation period of 54 days (Fig. 1). Tumors of TAP-treated mice grew faster than those of control mice during the first 2 weeks of treatment and subsequently underwent progressive regression until the end of treatment. Both growth-enhancing and reversing effects of the agonist were dose-dependent: tumors grew and regressed more rapidly at 600 μg than at 300 μg. The ovarian weight was significantly smaller in both treated groups than in the control ($P < 0.01$), but the same in both treated groups (Table 1). Morphologically, the ovaries from control mice had many apparently active corpora lutea and follicles of various developmental stages (Fig. 2a). In contrast, marked atrophy of the luteal components occurred in TAP-treated mice (Fig. 2b): there were a few solid, active corpora lutea in some ovaries treated with 300 μg, but none in those treated with 600 μg. However, the agonist had no significant influence on folliculogenesis at either dose. Thus, the decreased ovarian weight was ascribable to the specific atrophy of the luteal components caused by the agonist. Mammary gland development in treated mice was not suppressed to such a degree as expected from the ovarian weight loss and tumor regression (Table 2). Generally, mammary glands were comparable to mid to late-pregnant glands (grades 3–4) in control mice (Fig. 2c) and to virgin to mid-pregnant glands (grades 2–3) in treated mice (Fig. 2d). The uterine weight was significantly higher in the group treated with 300 μg than in the control (Table 1). This may be explained by the selective suppressive effect of the agonist on P

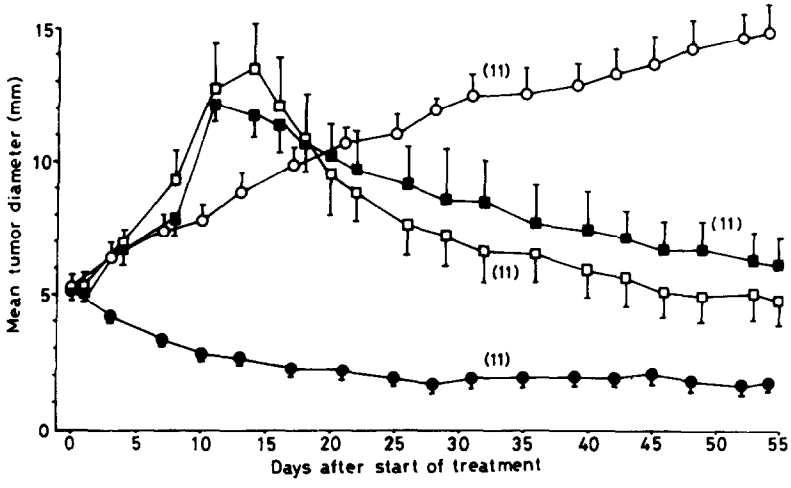

Fig. 1. Effect of TAP-144 on growth of TPDMT-4 pregnancy-dependent mammary tumors in PI-bearing mice. ○, *Control,* 0.1 ml *saline solution 6 times weekly;* □, 600 μg *TAP-144 6 times weekly;* ■, 300 μg *TAP-144 6 times weekly;* ●, *ovariectomized on day 0 and* 0.1 ml *saline solution 6 times weekly; number in parentheses, number of mice included; bar S.E.*

Table 2. Effect of TAP-144 on mammary gland development in Experiments 1 and 2

Experiment	Treatment	No. (%) of mice with glands of grade:* 0	1	2	3	4
1	Saline solution	0(0)	0(0)	1(9.1)	3(27.3)	7(63.6)
	TAP-144, 600 μg	0(0)	1(9.1)	3(27.3)	7(63.6)	0(0)
	TAP-144, 300 μg	0(0)	1(9.1)	2(18.2)	6(54.5)	2(18.2)
	Ovariectomy	4(36.4)	7(63.6)	0(0)	0(0)	0(0)
2	Ovariectomy	1(16.7)	5(83.3)	0(0)	0(0)	0(0)
	Ovariectomy + TAP-144, 600 μg	0(0)	6(100)	0(0)	0(0)	0(0)

*See References [17] and [19] for classification of mammary gland development.

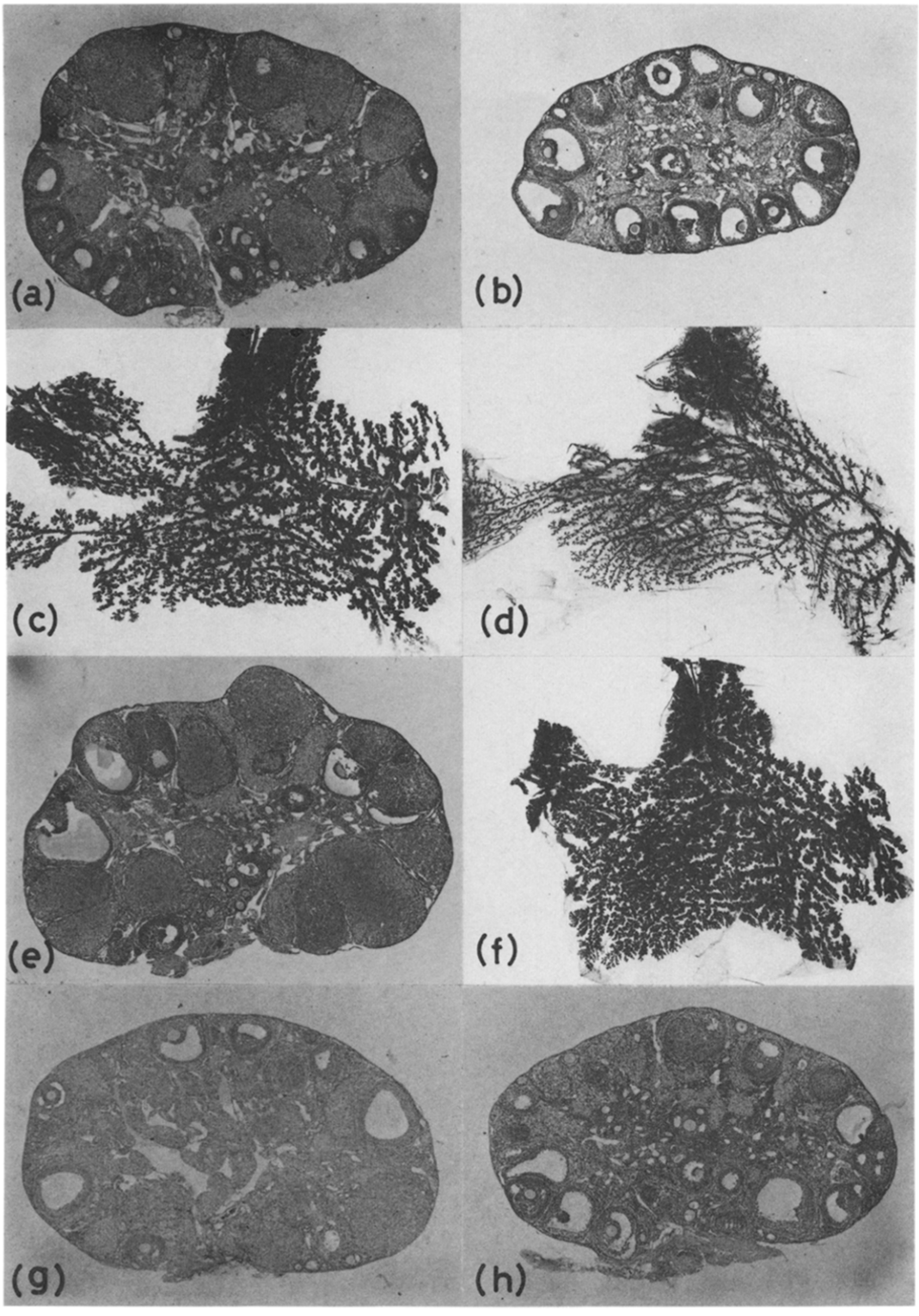

Fig. 2. Effect of TAP-144 on ovary and mammary gland. (a) Ovary from untreated PI-bearing mouse. Note many large, solid corpora lutea and follicles of various developmental stages; (b) ovary from PI-bearing mouse treated with 600 μg TAP-144 daily for 54 days. Note complete loss of luteal components but presence of follicles of various developmental stages, and size far smaller than that in (a); (c) thoracic mammary gland (grade 4) from untreated, PI-bearing mouse. Note full lobuloalveolar development comparable to late-pregnant status; (d) thoracic mammary gland (grade 3) from PI-bearing mouse treated with 600 μg TAP-144 for 54 days. Note less-developed lobules than those in (c); (e) ovary from PI-bearing mouse treated with 600 μg TAP-144 daily for consecutive 8 days. Note a greater number of more solid corpora lutea than in (a); (f) thoracic mammary gland (grade 4+) from PI-bearing mouse treated with 600 μg TAP-144 daily for 8 days. Note more highly developed lobules and less empty spaces than in (c); (g) ovary from untreated virgin mouse. Note a few solid corpora lutea and follicles of various developmental stages; (h) ovary from virgin mouse treated with 600 μg TAP-144 daily for 44 days. Note almost completely degenerated luteal components but survived follicles of various developmental stages. H and E, ×26 in (a), (b), (e), (g) and (h); hematoxylin, ×2.6 in (c), (d) and (f).

production by corpora lutea which was morphologically confirmed, since P inhibits E-induced growth of the uterus [20]. TAP-144 had no influence on the adrenal weight (Table 1). In order to investigate the effect of short-term treatment on the ovary and mammary gland, an additional group of 6 PI-bearing mice with palpable tumors were treated with 600 μg TAP-144 daily for 8 days. Enhancement of tumor growth was reproduced during this period (data not shown). Their ovaries had a greater number of more solid corpora lutea as compared with control ovaries (Fig. 2e). All mice had fully developed mammary glands (grade 4+) which were comparable to late-pregnant glands and composed of well-formed, thicker lobules of enlarged alveoli and less empty spaces (Fig. 2f). The morphology suggests active production of prolactin by PI.

Effect of TAP-144 on regression of TPDMT-4 tumors following ovariectomy in PI-bearing mice (Experiment 2)

Tumors regressed following ovariectomy in the same course in the control group and in the group treated with 600 μg TAP-144 (data not shown) (Table 1). The agonist promoted neither tumor growth nor regression in the absence of ovaries. It also did not affect mammary gland development under this condition (Table 2). The uterine weight was significantly larger in the treated group (Table 1), although the reason is not clear.

Effect of TAP-144 on growth of TPDMT-4 tumors induced by an EP pellet (Experiment 3)

Experiments 1 and 2 clearly validated the indispensability of the ovary for expression of the dual effects of TAP-144. This experiment was conducted to investigate whether the agonist displays similar effects on the tumor growth induced by an EP pellet as on that induced by PI. The agonist did not affect the tumor growth for the first 10 days, but subsequently enhanced it in a dose-related fashion until the end of 32 days of treatment (data not shown). Thus, the final tumor weight and diameter were larger, in the order: 600 μg, 300 μg, and control groups (Table 1). No mice underwent tumor regression during the course of treatment.

Effect of TAP-144 on growth of TPDMT-4V tumors in virgin mice (Experiment 4)

Experiment 3 indicates that TAP-144 enhances tumor growth in the presence of E and P without PI. It is, however, unknown whether the agonist also causes tumor regression in the absence of PI. The TPDMT-4V tumor, an ovarian-dependent subline isolated from a TPDMT-4 tumor, was used to clarify this issue. As shown in Fig. 3, 600 μg TAP-144 enhanced tumor growth for the first 10 days and subsequently caused tumor regression, although both growth-enhancing and reversing effects were not as conspicuous as in the parent tumors of PI-bearing mice (Fig. 1). In addition, tumors grew far more slowly than the parent tumors did in control mice. These findings are probably related to the lack of additional stimulation from PI. The selective suppressive effect of TAP-144 on the luteal components of the ovary was reconfirmed morphologically: the control ovaries had a few solid corpora lutea (Fig. 2g), but the treated ones lacked them (Fig. 2h). The relatively smaller uterine weight in the treated group than in the control (Table 1) suggests that the agonist might suppress E production to some degree at large doses.

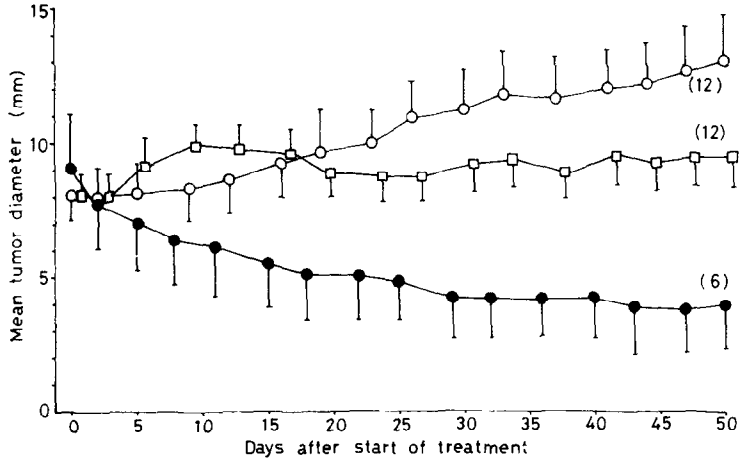

Fig. 3. Effect of TAP-144 on growth of TPDMT-4V ovarian-dependent mammary tumors in virgin mice. ○, *Control,* 0.1 ml *saline solution 6 times weekly;* □, 600 μg *TAP-144 6 times weekly;* ●, *ovariectomized on day* 0 *and* 0.1 ml *saline solution 6 times weekly; number in parentheses, number of mice included; bar, S.E.*

Effect of TAP-144 on regrowth of TPDMT-4 tumors induced by ovarian grafts in ovariectomized, PI-bearing mice (Experiment 5)

Experiments 1–4 have provided circumstantial evidence indicating that TAP-144 stimulates the PI and pituitary gland *in situ* to secrete hormones which enhance tumor growth in a direct way and/or via the ovary. This experiment was conducted to determine whether prolonged treatment of PI and pituitary gland *in situ* with the agonist influences its dual effects on tumor growth. As indicated in Fig. 4, the tumor regression following ovariectomy was not affected at all, as confirmed in Experiment 2. Progressive regrowth of regressed tumors occurred after ovarian grafting in control mice. The biphasic behavior of tumor growth under the influence of TAP-144 in Experiment 1 (Fig. 1) was reproduced after ovarian grafting in two treated groups, although significant tumor regrowth was not recorded in 2 mice of each group. Either growth-enhancing or reversing action was far greater in degree when TAP-144 treatment was commenced on the day of ovarian grafting than when commenced on the day of ovariectomy (30 days before ovarian grafting).

Although accurate ovarian weight determination was impossible because of difficulty in trimming adhering fat and connective tissue off the grafted ovaries, the weight was significantly smaller in the two treated groups than in the control ($P < 0.01$; mean ± S.E.: 19.5 ± 1.6 mg in control; 11.7 ± 1.1 mg in group treated from day of ovariectomy; 10.8 ± 0.7 mg in group treated from day of ovarian grafting). The ovarian grafts from the control and treated mice presented practically the same morphology as that of the comparable ovaries from Experiment 1.

Tumor morphology

Growing tumors from untreated, PI-bearing mice showed similar morphology to those described in [11, 16, 17]. They consisted of small cuboidal epithelial cells which were arranged in multiple layers or in single rows, forming acinar and glandular spaces of various sizes filled with secretion. Regressing tumors from ovariectomized or TAP-144-treated mice showed the same morphology as described in [16, 17]. They were characterized by clusters of simple tubules of various diameters. Growing tumors from mice given an implant of an EP pellet alone or followed by TAP-144 treatment were the same in morphology and composed of small cuboidal epithelial cells which were more densely scattered with hyperplastic tubules at the periphery. Interestingly, the most rapidly growing tumors from PI-bearing mice treated with 600 μg TAP-144 for 8 days were more similar to those tumors in closer arrangement of cells and less secretion than to tumors from untreated, PI-bearing mice.

The morphology of growing TPDMT-4V tumors was similar to that of growing parent

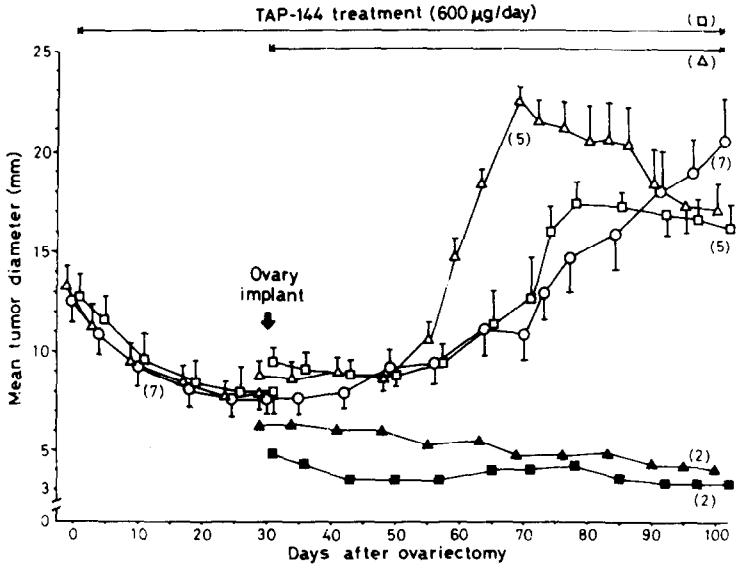

Fig. 4. Effect of TAP-144 on regrowth of TPDMT-4 tumors induced by ovarian grafts in ovariectomized, PI-bearing mice. Mice with palpable tumors were ovariectomized on day 0 and received an implant of ovarian grafts on day 30. ○, Control, 0.1 ml saline solution per day from day 0 on; △, 0.1 ml saline solution per day on days 0–29 and 600 μg TAP-144 from day 30 on; □, 600 μg TAP-144 per day from day 0 on; number in parentheses, number of mice included; bar, S.E. Mice which did not produce significant regrowth of tumors after ovarian grafting are presented separately (▲, ■).

tumors at later transplant generations [19]. Small cuboidal epithelial cells also composed the subline tumors, but they were arranged in 2 or more layers and lined angular spaces or fissures with occasional formation of papillary projections. Most lumina were devoid of secretion. Regressed or static tumors after ovariectomy or TAP-144 treatment had similar histological structures, but tended to have more tubules formed by single-layered cells, larger empty spaces and more stroma than growing tumors.

DISCUSSION

Several reports have indicated that GnRH agonist analogs caused regression of spontaneous and DMBA-induced, hormone-dependent rat mammary carcinomas and prevented the development of new tumors [6–9, 21]. DMBA-induced rat mammary tumors have been extensively used as a model for study on endocrine therapy of breast cancer, and their growth is known to be under the control of E and prolactin [22]. Thus, the mechanism of their antitumor action has been proposed at both levels. DeSombre *et al.* [8] found TAP-144 treatment to be as effective as ovariectomy in causing regression of DMBA-induced rat mammary tumors and ascribed its antitumor activity to the production of a temporary chemical ovariectomy. In support of this conclusion, the multiple direct inhibitory effects of GnRH and its agonists on the ovarian functions have been demonstrated *in vivo* [4, 5, 23] and *in vitro* [4, 5, 24–26]. The presence of specific, high-affinity receptor sites for GnRH and its agonists in the ovary has provided additional evidence for their direct interference with ovarian functions [25–28]. On the other hand, Danguy *et al.* [7] indicated that TAP-144 exerted an antitumor effect on this model system by causing atrophy of pituitary lactotropes and reducing the plasma prolactin concentration without apparent suppression of ovarian E secretion. More recently, Rose and Pruitt [9] have indicated that growth suppression by TAP-144 was prevented by administration of either E or perphenazine, a plasma prolactin-increasing agent, in DMBA-induced rat mammary tumors which are dependent on both pituitary and ovary, whereas it was prevented by the former but not by the latter in *N*-nitrosomethylurea-induced rat mammary tumors which are dependent on the ovary alone, suggesting implication of both mechanisms in the antitumor action of GnRH agonists in this animal species.

Corbin *et al.* [29] have observed retardation of the growth of mouse mammary tumors by a GnRH agonist in 4-day-old hamsters immunologically suppressed with anti-lymphocyte serum. In the present study, however, TAP-144 enhanced the growth of TPDMT-4 mouse mammary tumors for the first 2 weeks of treatment and subsequently gave rise to rapid regression in PI-bearing syngeneic hosts (Fig. 1). The unexpected, growth-enhancing effect was not ascribed to the presence of ectopic PI since the agonist also stimulated the growth of TPDMT-4V tumors for the same period in normal virgin mice (Fig. 3). E, P and pituitary hormones, primarily prolactin, are important for growth of these tumors [11, 13]. TPDMT-4 tumors can grow without regression in ovariectomized, EP pellet-carrying mice [17]. TAP-144 exerted only a growth-enhancing effect in this system (Table 1; Experiment 1), suggesting that it might elevate the prolactin level continuously. In fact, TAP-144 treatment for 20 or 50 days significantly increased the hormone level at a daily dose of 500 μg, which is equivalent to those used in the current study [30]. Stimulated tumors from PI-bearing mice on day 8 of treatment with the agonist were similar to those from late-pregnant mice [10] and EP pellet-carrying mice [16, 17] in morphological characteristics, and their hosts had maximally developed mammary glands (Fig. 2c) and the largest ovaries with a greater number of more solid corpora lutea (Fig. 2d). In addition, the agonist did not cause tumor growth in the absence of ovaries in PI-bearing mice (Table 1; Experiment 2, Fig. 4). Taking these results together, it is conceivable that TAP-144 elevated the blood hormone levels and enhanced tumor growth during an early period of treatment by stimulating the PI and pituitary gland *in situ* to produce gonadotropic hormones and prolactin, which in turn stimulated the ovaries to produce E and P. In ovariectomized, PI-bearing mice, ovarian grafts gave rise to progressive regrowth of the regressed tumors. In this system the agonist was less effective in both enhancing and reversing tumor regrowth when its administration was started before ovarian grafting (Fig. 4). This suggests that longer exposure to the agonist may make the pituitary gland less sensitive to its stimulative action. A similar phenomenon was observed for plasma LH release using another agonist in rats [31].

Mice with tumors regressing under the influence of TAP-144 had significantly smaller ovaries than the respective controls (Table 1; Experiments 1, 4). Morphologically, they were characterized by selective atrophy of the luteal

components (Figs 2b, 2h). This effect was dose-dependent and unrelated to the presence of PI, suggesting that the agonist may cause ovarian atrophy through its direct action, as evidenced in rats [8]. Ovarian grafts caused enlargement of atrophied uteri but not regrowth of regressed tumors under the influence of TAP-144 in 4 ovariectomized, PI-bearing mice (Fig. 4). Although the reason is unclear, the direct inhibitory effect of the agonist on the luteal cells was probably predominantly expressed from the beginning of treatment in these mice. A similar selective effect on corpora lutea has been reported in both rats and mice [3, 30]. Although the agonist had morphologically no influence on folliculogenesis (Figs. 2b, 2c), reduction in uterine weight by daily doses of 600 μg in virgin mice (Table 1; Experiment 4) is indicative of its ability to suppress E production at large doses.

TAP-144 causes hormone-dependent mammary tumors to regress through its inhibitory effect on ovarian functions in both mouse and rat experimental model systems. However, this effect is preceded by a short period of significantly enhanced tumor growth, probably via the pituitary–ovarian axis, in the mouse. This discrepant result may be related to the different sensitivities to the agonist of the two animal species on the one hand, and to the different mechanism of hormonal control of the neoplastic growth on the other. In this respect it is noteworthy that the effective dose is 30 to 50-fold higher in the mouse and that progesterone is essential for tumor growth in the mouse system but not in the rat. In clinical application of GnRH agonist analogs attention should be paid to the tumor growth-enhancing effect confirmed in the current study.

Acknowledgements—We are grateful to Dr. Y. Sugino, Takeda Chemical Industries, Ltd. (Osaka, Japan), who kindly gifted TAP-144. We are indebted to Mr. T. Kaneko for technical assistance.

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