20. Maheshware RK, Lazo PS, Friedman RM. Enhancement of interferon activity by membrane glycoprotein related to the thyrotropin receptor. In: Khan S, Hill NO, Dorn GL, eds. *Interferon: Properties and Clinical Uses.* Dallas, Leland Fikes Foundation Press of the Wadley Institute of Molecular Medicine, 1979, 387–396.
21. Blalock JE, Smith EM. Human leukocytes interferon: potent endorphin-like opioid activity. *Biochem Biophys Res Commun* 1981, **101**, 472–478.
22. McDonald EM, Mann AH, Thomas HC. Interferons as mediators of psychiatric morbidity. *Lancet* 1987, ii, 1175–1178.
23. Kirkwood JM, Harris JE, Vera R *et al.* A randomized study of low and high doses of leukocyte alpha interferon in metastatic renal cell carcinoma: the American Cancer Society collaborative trial. *Cancer Res* 1985, **45**, 863–871.

Eur J Cancer, Vol. 26, No. 5, pp. 600–603, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Effect of CGS 16949A plus Tamoxifen on Induced Mammary Tumours in Rats

T. Tominaga, Y. Yoshida, K. Shimozuma, K. Hayashi and G. Kosaki

The antitumour effect of CGS 16949A, an aromatase inhibitor, was investigated in rats with mammary tumours induced by 7,12-dimethylbenz[a]anthracene. A dose-dependent antitumour effect was observed after daily oral administration of CGS 16949A for 3 weeks. The tumour did not recur in the groups treated with 4.0 and 8.0 mg/kg per day. The complete remission rate increased and the time required to achieve complete remission became shorter with increasing daily doses. After daily administration for 3 weeks, a significant antitumour effect was observed in the group treated with CGS 16949A plus tamoxifen compared with that seen either with CGS 16949A or with tamoxifen alone. At the end of treatment, the group treated with CGS 16949A had significantly decreased oestradiol-17β and prolactin levels and increased levels of follicle stimulating hormone, but oestrone was not affected.
Eur J Cancer, Vol. 26, No. 5, pp. 600—603, 1990.

INTRODUCTION

THE ROLE of oestrogens in the initiation and promotion of breast cancer has long been recognized [1]. The growth of breast cancer can also be inhibited by the deprivation of oestrogens via blockade of synthesis of oestradiol or its precursors [2]. Endocrine therapy has been widely used to reduce the amount of oestradiol acting locally on the tumour. Gonadotropin-releasing hormone (GnRH) analogues [3–5] which inhibit the release of LH and FSH, and aromatase inhibitors [6, 7], which inhibit steroid biosynthesis, are attracting attention.

Similarly to several other steroidogenic enzymes, aromatase is cytochrome P-450 dependent [8]. The first, clinically useful aromatase inhibitor, aminoglutethimide, not only reduces the amount of circulating oestrogens by inhibiting aromatase but also inhibits adrenal steroidogenesis. It is also an androgen antagonist [9]. Trials of aminoglutethimide to block aromatase in postmenopausal patients with breast cancer showed efficacy similar to that following surgical adrenalectomy, but central nervous system and dermatological side-effects are substantial [10]. Because of aminoglutethimide's action, glucocorticoids have to be co-administered.

CGS 16949A is a nonsteroidal competitive inhibitor of aromatase that has shown higher potency and greater specificity in inhibition of aromatase than aminoglutethimide [6, 11]. We have studied the antitumour effect of CGS 16949A alone and combined with tamoxifen in rats with oestrogen-dependent mammary tumours.

Correspondence to: T. Tominaga, Department of Surgery, Tokyo Metropolitan Komagome Hospital, 3–18–22, Honkomagome, Bunkyo-ku, Tokyo, Japan 113.
T. Tominaga, Y. Yoshida, K. Shimozuma, K. Hayashi and G. Kosaki are at the Department of Surgery, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan.

MATERIALS AND METHODS

Female Sprague–Dawley rats about 8 weeks old and weighing 180–200 g were used. 7,12-Dimethylbenz[a]anthracene (7,12-DMBA) was dissolved in olive oil (20 mg/ml), and was orally administered to 120 rats as a single dose of 100 mg/kg. After 8–12 weeks, 70 rats, whose mammary tumours measured 0.8–1.2 cm at their widest diameter, were selected and used for the experiment [12].

Tumour size and body weight were measured before treatment and weekly thereafter. Drugs were administered orally once daily for 3 weeks. Animals received a suitable diet (F2) and water freely.

At the end of treatment and at the end of the experiment (6 weeks), the animals were killed and the ovaries, uterus and adrenals were removed and weighed. At the same time, serum levels of oestradiol-17β, oestrone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) and prolactin were measured by radioimmunoassay.

Tumour size (cm^2) was defined as the product of the widest diameter and the greatest diameter perpendicular to it, and was expressed as the percentage of the initial size measured on day 0.

CGS 16949A was supplied by Ciba-Geigy and tamoxifen by ICI. CGS 16949A was dissolved in physiological saline and tamoxifen was suspended in 5% sodium carboxymethylcellulose (CMC-Na).

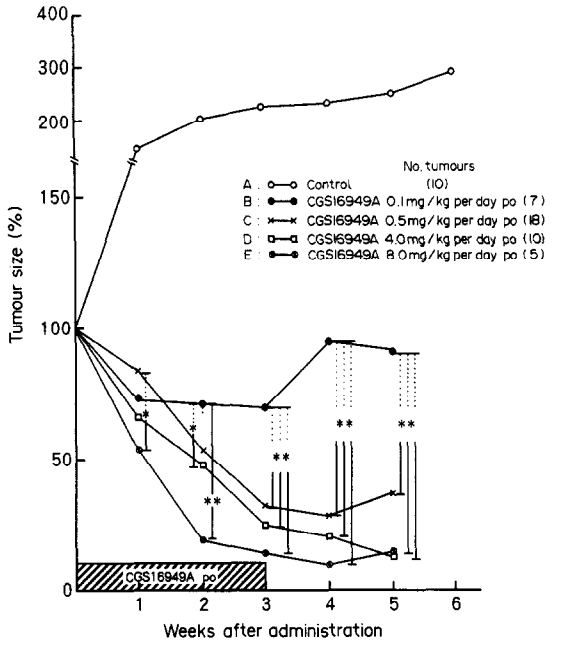

Fig. 1. Effect of CGS 16949A on rat mammary tumours induced by 7,12-DMBA. Tumour size of each treated group was significantly smaller than that of controls at all points ($P < 0.001$). Significant differences between groups: *$P < 0.05$, **$P < 0.01$.

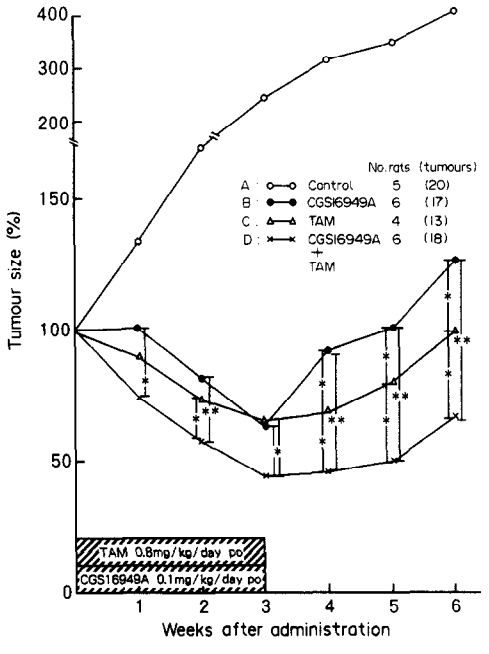

Fig. 2. Effect of tamoxifen and CGS 16949A on rat mammary tumours. Tumour size of each treated group was significantly smaller than that of controls at all points ($P < 0.001$). Between groups: *$P < 0.05$, **$P < 0.01$.

In a preliminary experiment, body weight and tumour size were compared in animals given one of the following regimens of solvents for 3 weeks: (1) oral administration of 2.0 ml/kg of physiological saline; (2) oral administration of 2.0 ml/kg of 5% CMC-Na suspension; (3) saline and CMC-Na; or (4) no treatment. Body weight and tumour size were similar among all groups, and it was also confirmed that serum hormone levels remained unchanged before and after the treatment.

RESULTS

Effect of CGS 16949A

The control group received 2.0 ml/kg per day of physiological saline. Following oral daily administration of 0.1, 0.5, 4.0 and 8.0 mg/kg of CGS 16949A for 3 weeks, tumour size regressed to 71.2, 31.4, 27.3 and 13.2%, respectively, of the control size at the start of treatment (Fig. 1) ($P < 0.01$ in all groups compared with control, t test). At 5 weeks tumour size was still reduced: 91.3, 37.2, 13.1 and 14.5%, respectively, of the control size ($P < 0.01$).

*Table 1. Complete remissions on rat mammary tumours induced by 7,12-DMBA after oral administration of CGS 16949A for 3 weeks**

Dose (mg/kg)	Total tumours	No. complete remissions at (weeks): 1	2	3	4	5
0	10	0	0	0	0	0
0.1	7	0	0	0	0	2 (29%)
0.5	18	0	0	1 (6%)	4 (22%)	4 (22%)
4.0	10	1 (10%)	2 (20%)	3 (30%)	4 (40%)	6 (60%)
8.0	5	1 (20%)	2 (40%)	4 (80%)	4 (80%)	4 (80%)

*5 rats per group.

Table 1 shows the complete remission rate (number of tumours that disappeared/total number of tumours) after administration of CGS 16949A. The rate increased and the time required to achieve complete remission became shorter with increasing doses.

CGS 16949A plus tamoxifen

Animals were assigned to: (1) control; (2) CGS 16949A at 0.1 mg/kg per day; (3) tamoxifen at 0.8 mg/kg per day; and (4) CGS 16949A and tamoxifen combined at these doses. Oral treatment lasted 3 weeks. At 3 and 6 weeks tumour size in the CGS 16949A group was 63.6 and 128.7%, respectively, of the pretreatment value (Fig. 2). The corresponding figures in the tamoxifen group were 65.1 and 99.2% and, in the combined treatment group, 44.9 and 66.2%. Combined administration had a significantly greater inhibitory effect on tumour growth compared with the corresponding single drug group.

Uterus weights were decreased significantly in the CGS 16949A (mean 368.4 [S.E. 9.10] mg, $n = 6$) and tamoxifen groups (348.2 [17.8] mg, $n = 5$) ($P < 0.05$), and more so in the combined treatment group (307.2 [11.4] mg, $n = 6$) ($P < 0.01$) compared with controls (491.5 [44.7] mg, $n = 6$). However, at 6 weeks uterine weights in the treated groups regained levels similar to those of the control group. Ovary weights at 3 weeks did not differ between the groups: 71.5 (5.4) mg for controls, 63.5 (1.3) mg for the CGS 16949A group, 70.6 (2.2) mg for the tamoxifen group, and 76.9 (3.5) mg for the combination group. However, ovarian weights were decreased significantly at 6 weeks (i.e. 3 weeks after the end of treatment) in the treated groups: 74.2 (2.0) mg, 61.2 (4.0) mg, 48.5 (2.9) mg and 54.8 (4.4) mg for the control, CGS 16949A, tamoxifen and combination groups, respectively. Adrenal weights were decreased significantly at 3 weeks in the CGS 16949A (53.6 [2.3] mg, $P < 0.05$) and combination groups (52.0 [2.8] mg, $P < 0.01$) compared with the control group (64.2 [3.2] mg), but adrenal

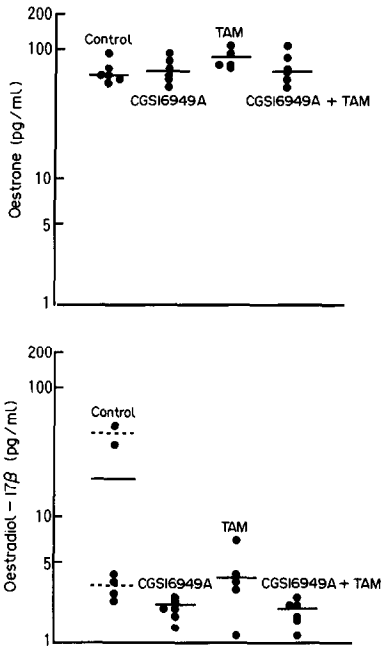

Fig. 3. Serum oestrone and oestradiol-17β after daily oral administration for 3 weeks of CGS 16949A 0.1 mg/kg and tamoxifen 0.8 mg/kg. For oestradiol-17β, CGS 16949A, alone and combined with tamoxifen, was significantly different from low level group of controls ($P < 0.01$).

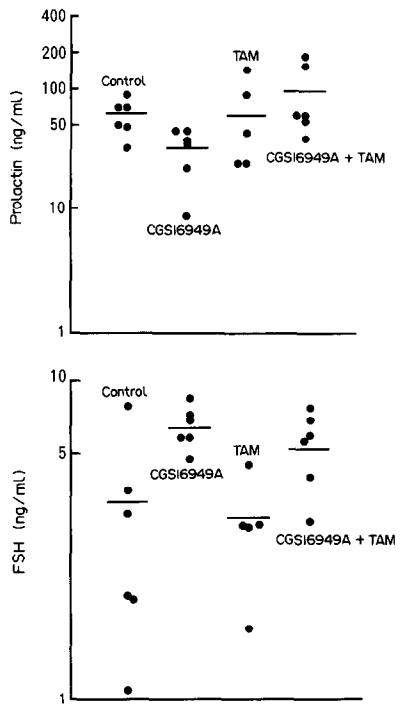

Fig. 4. Serum prolactin and FSH. For prolactin, CGS 16949A was significantly different from controls ($P < 0.05$). For FSH, CGS 16949A, alone ($P < 0.001$) or combined with tamoxifen ($P < 0.05$), was significantly different from controls.

weights regained the levels of control groups at 6 weeks, as did the uterine weights. Body weight was not affected in any group.

Serum oestradiol-17β levels in the controls were divided into low (3.08 [0.63] pg/ml, $n = 4$) and high level groups (54.95 pg/ml, $n = 2$) because of the oestrus cycle (Fig. 3). Serum oestradiol-17β in the CGS 16949A group (1.92 [0.32] pg/ml, $n = 6$) was decreased irrespective of the stage of oestrus and showed a significant difference ($P < 0.01$) even when compared with the low level group of controls. The CGS 16949A plus tamoxifen group also showed a decreased oestradiol-17β level (1.92 [0.44] pg/ml, $n = 6$). Significant decrease of prolactin level (control: 61.8 [8.82] ng/ml, CGS 16949A: 32.6 [5.82] ng/ml, $n = 6$, $P < 0.05$) and increase of FSH level (control: 3.12 [0.83] ng/ml, CGS 16949A: 5.92 [0.36] ng/ml, $n = 6$, $P < 0.01$) were observed when CGS 16949A was administered (Fig. 4); however, oestrone and LH levels were not changed.

DISCUSSION

The major source of circulating oestrogens in postmenopausal women is derived from the conversion of adrenal androgens to oestrogens by aromatase in peripheral tissues such as adipose tissue, including the breast [13]. Aromatase is a key enzyme for oestrogen biosynthesis, both in premenopausal and postmenopausal women.

In our study CGS 16949A inhibited the growth of 7,12-DMBA induced mammary tumours in a dose dependent way in rats. This compound also increased the frequency of complete remissions, the time needed to achieve such a remission gradually becoming shorter with increasing daily doses. Schieweck *et al.* [11] reported that the frequency of the same tumours in Sprague–Dawley rats was decreased dose-dependently by oral administration of CGS 16949A for 42 consecutive days. Steele *et al.* [6] showed that CGS 16949A was about 400 times more potent an inhibitor of aromatase and more selective in action than aminoglutethimide.

We found that serum oestradiol-17β levels were decreased irrespective of stages of oestrus following oral daily administration of CGS 16949A 0.1 mg/kg for 3 weeks to rats with mammary tumours, even allowing for the oestrus stage of the controls. However, serum oestrone levels were decreased in women similarly to oestradiol-17β levels [14] while in rats no difference was found compared with controls. Whether human aromatase or the human metabolic system differs from that in rats would be interesting to study. The uterine weights of the animals treated with CGS 16949A also decreased significantly compared with those of the control group. The decrease in serum oestradiol-17β levels and uterine weights of this group agrees with *in vitro* and *in vivo* observations of rat uterus [6]. This result is attributed to the decreased production of oestrogens resulting from CGS 16949A influenced aromatase inhibition in the ovaries.

Tamoxifen and CGS 16949A differ in their mechanism of action. As a result of combined endocrinological therapy, tumour growth was significantly inhibited compared with the corresponding single drug treatments. Although serum FSH levels were increased significantly at 3 weeks in the CGS 16949A and CGS 16949A plus tamoxifen groups compared with controls (LH was not affected), oestradiol-17β levels in these two groups were decreased. This suggests that antitumour action can be obtained by decreasing the amount of oestradiol-17β in the serum (i.e. the amount of oestradiol acting locally on the tumour) by combined endocrinological therapy with drugs whose mechanisms of action differ.

For aminoglutethimide, survival at 4 weeks after the start of treatment was low at 67%, due to the occurrence of side-effects (even when 2.4 mg/kg per day of aminoglutethimide was administered with hydrocortisone). At this dose, aminoglutethimide did not change tumour size when administered orally for 3 weeks [15].

At the dose we used, CGS 16949A did not cause side-effects. CGS 16949A is a useful oral aromatase inhibitor which would be expected to have an excellent anti-tumour effect, especially

when used in combination with other endocrinologically acting drugs with different mechanisms of action.

1. Beatson GT. Suggestions for a new method of treatment with illustrative cases. *Lancet* 1896, ii, 104–107.
2. Santen RJ. Novel methods of oestrogen deprivation for treatment of breast diseases. *Proc R Soc Edinburgh* 1989, **95B**, 255–269.
3. Plowman PN, Walker KJ, Nicholson RI. Remissions of postmenopausal breast cancer during treatment with luteinizing hormone agonist ICI 118630. *Br J Cancer* 1986, **54**, 903–909.
4. Schally AV, Redding TW, Comaru-Schally AM. Potential use of analogues of luteinizing hormone-releasing hormone in the treatment of hormone-sensitive neoplasms. *Cancer Treat Rep* 1984, **68**, 281–289.
5. Santen RJ, Manni A, Harvey H. Gonadotropin releasing hormone (GnRH) analogs for the treatment of breast and prostatic carcinoma. *Breast Cancer Res Treat* 1986, **7**, 129–145.
6. Steele RE, Mellor LB, Sawyer WK, Wasvary JM, Browne LJ. *In vitro* and *in vivo* studies demonstrating potent and selective oestrogen inhibition with the nonsteroidal aromatase inhibitor, CGS 16949A. *Steroids* 1987, **50**, 147–161.
7. Santen RJ, Santen S, Davis S, Veldhuis J, Samojlik E, Ruby E. Aminoglutethimide inhibits extraglanduar oestrogen production in postmenopausal women with metastatic breast carcinoma. *J Clin Endcrinol Metab* 1978, **47**, 1257–1265.
8. Thompson EAJ, Siiteri PK. The involvement of human placental microsomal cytochrome P-450 in aromatization. *J Biol Chem* 1974, **249**, 5373–5378.
9. Dexter RN, Fishman LM, Ney RL, Liddle GW. Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action. *J Clin Endocrinol Metab* 1967, **27**, 473–480.
10. Brodic AM, Santen RJ. Aromatase in breast cancer and the role of aminoglutethimide and other aromatase inhibitors. *CRC Crit Rev Oncol/Hematol* 1986, 361–396.
11. Schieweck K, Bhatnagar AS, Matter A. CGS 16949A, a new nonsteroidal aromatase inhibitor: effects on hormone-dependent and-independent tumors *in vivo*. *Cancer Res* 1988, **48**, 834–838.
12. Tominaga T, Yoshida Y, Kitamura M, Kosaki G. Effective sequential administration of tamoxifen and medroxyprogesterone acetate for 7,12-dimethylbenz[a]anthracene-induced rat mammary tumors in relation to hormone receptors. *Jpn J Cancer Res* 1985, **76**, 1120–1125.
13. Grodin JM, Siiteri PK, MacDonald PC. Source of oestrogen production in the postmenopausal woman. *J Clin Endocrinol Metab* 1973, **36**, 207–214.
14. Santen RJ, Demers LM, Adlecreutz H *et al.* Inhibition of aromatase with CGS 16949A in postmenopausal women. *J Clin Endocrinol Metab* 1989, **68**, 99–106.
15. Tominaga T, Yoshida Y, Kitamura M, Kosaki G. Effect of combined administration of aminoglutethimide, tamoxifen and medroxyprogesterone acetate on rat mammary tumors induced by 7, 12-DMBA. The 46th Proceedings of the Japan Cancer Association, 1987.

Eur J Cancer, Vol. 26, No. 5, pp. 603–608, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Interactions between Growth Factor Secretion and Polyamines in MCF-7 Breast Cancer Cells

Fredric J. Cohen, Andrea Manni, Patricia Glikman, Mary Bartholomew and Laurence Demers

Polyamines may be involved in hormone-dependent breast cancer cell proliferation. The antiestrogen 4-hydroxytamoxifen and the polyamine synthesis inhibitor α-difluoromethylornithine (DFMO) inhibited MCF-7 growth, and this effect was additive. Transforming growth factor β (TGF-β) levels were increased by both compounds; again the effect was additive. Exogenous putrescine antagonized DFMO but not the antiestrogen. However, exogenous TGF-β did not inhibit cell growth. Secretion of insulin-like growth factor 1 (IGF-1) was not affected by DFMO-induced polyamine depletion but 4-hydroxytamoxifen increased IGF-1, which suggests an estradiol-like effect. Thus polyamines are involved in basal TGF-β secretion but do not mediate antiestrogen-induced TGF-β secretion. IGF-1 secretion by MCF-7 cells is not under polyamine control. The antiproliferative effects of 4-hydroxytamoxifen and DFMO cannot be accounted for by either suppression of IGF-1 secretion (a growth stimulatory factor) or stimulation of TGF-β production (a growth inhibitory polypeptide).
Eur J Cancer, Vol. 26, No. 5, pp. 603—608, 1990.

INTRODUCTION

STEROID HORMONES may regulate hormone-responsive breast cancer cell proliferation, at least in part, via the production and secretion of various polypeptide growth factors that alter cell growth [1, 2]. Conversely antagonists such as tamoxifen may inhibit breast cancer growth by suppressing hormone-stimulated growth factor production [2] and/or by increasing the secretion of polypeptides, such as transforming growth factor-β (TGF-β), which are thought to inhibit growth effect of breast cancer cells [3]. Putrescine, spermidine, and spermine are essential mediators of hormonally stimulated breast cancer cell growth *in vitro* [4–7] interacting with autocrine/paracrine effectors of such growth [8]. The polyamine pathway may also be involved in antiestrogen-mediated inhibition of breast cancer cell growth *in vitro* [9] and *in vivo* [10].

We have evaluated the role of polyamines in antiestrogen-regulated secretion of insulin-like growth factor 1 (IGF-1) and TGF-β by MCF-7 breast cancer cells in culture.

Correspondence to: A. Manni, Division of Endocrinology, Milton S. Hershey Medical Center, P.O. Box 850, Hershey, PA 17033, U.S.A. F.J. Cohen, A. Manni, P. Glikman, M. Bartholomew and L. Demers are at the Departments of Medicine and Pathology and Center for Biostatistics and Epidemiology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania, U.S.A.