ABSTRACT

A gonadoliberin analog, (D-leucyl6, desglycyl-NH210, prolyl ethylamide9) gonadoliberin, is known to suppress ovarian function and plasma prolactin levels. Its antitumor activity was evaluated against mammary tumors induced in Sprague-Dawley rats by dimethylbenz(a)anthracene. Observations were made when the analog, referred to as A-43818, was given alone and together with estrogen replacement or perphenazine. A-43818, 10 μg s.c. twice a day for 6 weeks, was highly effective in producing tumor remissions. All of the 11 animals survived throughout the observation period, complete regressions occurred in 8 of 13 tumors, and 2 were classified as static. None of the 16 tumors in 12 control rats regressed, and there were 4 deaths. When estradiol benzoate, 2 μg s.c. each day, was administered with the A-43818, antitumor activity was suppressed; only 2 of 17 tumors regressed, 6 were static, and 5 of the 10 rats in this group died. Perphenazine, 1 mg i.m. daily, a dose known to cause hyperprolactinemia, also impaired the efficacy of A-43818. Three of 14 tumors regressed, 6 were static, and the rest continued to grow; 3 of the 12 rats died within 6 weeks of starting treatment.

INTRODUCTION

A series of analogs of gonadoliberin (luteinizing hormone-releasing hormone or gonadotropin-releasing hormone) has been synthesized, each having agonistic or antagonistic hormonal properties. One of these, (D-leucyl6, desglycyl-NH210, prolyl ethylamide9) gonadoliberin, prepared by Abbott Laboratories (North Chicago, Ill.) and allocated by them the reference code A-43818, has antitumor activity against DMBA3-induced rat mammary tumors (3, 4). A-43818 is 3 to 5 times more potent than gonadoliberin in stimulating the release of luteinizing hormone and follicle-stimulating hormone from rat pituitary in vitro (6). Chronic administration of the analog to mature female rats, although associated with sustained elevations in serum luteinizing hormone and follicle-stimulating hormone, causes cessation of the estrus cycle and atrophy of the ovaries and uterus (7). These effects are ascribed to inhibition of ovarian hormone secretion.

DeSombre et al. (4) considered that the suppression of mammary tumor growth by A-43818 was due essentially to a "chemical ovarietomy." The DMBA rat mammary carcinomas are, however, primarily prolactin dependent (8), and Danguy et al. (3) concluded from their experiments that A-43818 exerted its antitumor effect by causing atrophy of pituitary lactotrophs, hence reducing the plasma prolactin concentration. The present study was designed to determine whether the therapeutic effects of A-43818 against DMBA tumors were inhibited when given in combination with a replacement dose of estrogen or by treatment with perphenazine, a drug known to induce hyperprolactinemia.

MATERIALS AND METHODS

The DMBA was prepared in a fat emulsion and was provided as a gift by The Upjohn Company (Kalamazoo, Mich.). Mammary tumors were induced in female Sprague-Dawley rats by administering a single 5-μg dose of DMBA i.p. when the animals were 50 days old. Tumor size was measured at weekly intervals with a caliper. Measurements of the maximum tumor diameter (L) and that at right angles to it (W) were made, and the surface area was calculated from the formula L/2 × W/2 × π. A-43818, a gift from Abbott Laboratories, was dissolved in 0.1% bovine serum albumin-0.9% NaCl solution at a concentration of 10 μg/0.5 ml. It was administered by s.c. injection in a dose of 10 μg twice daily at 8 a.m. and 4 p.m. Estradiol benzoate (Sigma Chemical Co., St. Louis, Mo.) was dissolved in corn oil, and 2 μg in 0.2 ml were given each day by s.c. injection. Perphenazine (Trilafon; Schering Corp, Bloomfield, N. J.), 1 mg in 0.2 ml, was administered by i.m. injection into a thigh muscle at 8 a.m.

Rats were allocated to 1 of 4 groups and matched as closely as possible for tumor size and age. The control group was given a twice-daily injection of 0.5 ml of the A-43818 solvent. The second group received A-43818, the third A-43818 plus estradiol benzoate, and the fourth A-43818 plus perphenazine. Treatment of all 4 groups was maintained for 6 weeks. After this period, some rats were subjected to further manipulations as described in "Results." Progression of tumor growth was defined as an increase of 50% or more in surface area and regression as a decrease of 50% or more; lesser changes were regarded as indicating a static situation. A tumor which ceased to be palpable was designated a complete regression.

RESULTS

The responses of animals in the control and 3 treatment groups are summarized in Table 1. The posttreatment surface area refers to the measurement after 6 weeks of treatment or the last measurement before death. The mean tumor area of the controls increased approximately 2-fold during the 6-week observation period. All the tumors in 7 of the 11 rats treated with A-43818 underwent complete remission, but the mean...
area for this group was unchanged because tumor growth progressed rapidly in 2 animals. The antitumor activity of A-43818 was inhibited both by replacement of endogenously produced estrogens and perphenazine administration. Thus, a statistical analysis using the Wilcoxon rank sum test showed that these 2 treatment groups and the controls all differed significantly in their tumor responses compared with the animals receiving A-43818 (p < 0.01 in each case; Table 1). The Kruskal-Wallis test was applied to the data to provide a comparison between more than 2 groups. There were no significant differences in the rates of tumor growth for the controls, the A-43818 plus estradiol benzoate, or A-43818 plus perphenazine-treated groups (p > 0.50). When these 3 groups were combined and compared with the animals treated only with A-43818, the difference in tumor responses was found to be highly significant (p < 0.001).

At the initiation of the study, some rats had more than 1 tumor, new tumors appeared during treatment, and 12 animals died within the 6-week observation period. Table 2 gives the responses of the individual tumors for each of the 4 groups; the therapeutic efficacy of A-43818 was again confirmed, in terms of both tumor regression and survival. Complete regressions occurred in 8 of 13 tumors, and all 11 animals were alive at the end of the 6 weeks. In contrast, 15 of the 17 tumors in the estrogen-supplemented group either progressed or remained static, and 5 rats died during treatment. A similar outcome occurred in the perphenazine-treated rats; 11 of the 14 tumors failed to regress during A-43818 administration, and there were 3 deaths.

One other rat was given A-43818 alone for 14 days, during which time the tumor surface area decreased by more than 50%. Perphenazine was then added to the treatment schedule and resulted in a rapid regrowth of the tumor. In the control group, 2 rats alive after 6 weeks of observation were then given A-43818; both responded to the analog. Four rats whose tumors had regressed during 6 weeks of treatment with A-43818 continued on therapy but also received 2 μg of estradiol benzoate for a further 3 weeks. In all 4 cases, tumor growth was stimulated by estrogen replacement (Chart 1).

**DISCUSSION**

Treatment of female rats with A-43818 alters both ovarian and pituitary function. Johnson et al. (7) observed an arrest of the estrus cycle in diestrus, with ovarian and uterine atrophy, in animals given 3 μg of the analog twice daily for 55 days. This effect was accompanied by elevations of plasma luteinizing hormone and follicle-stimulating hormone. In an unpublished study, we found that 10 μg of A-43818 given twice a day produced an acyclic state of constant diestrus within 2 weeks of starting treatment. Although the mechanism for these effects remains to be established, it may involve the loss of ovarian gonadotropin receptors. In male rats, treatment with this gonadolibehrin agonist causes inhibition of spermatogenesis and depletion of testicular luteinizing hormone-human chorioc gonadotropin receptors (2).

Two mechanisms have been proposed for the antitumor activity of A-43818. DeSambre et al. (4) found the analog to be as effective as ovariectomy in causing regressions of DMBA-induced rat mammary tumors, an effect which they ascribed to the production of a temporary chemical ovariectomy. In support of this conclusion, they quoted the previously reported suppressive effect of A-43818 on ovarian function and unpublished data which showed that this is accompanied by a reduction in serum estrogens and prolactin. Danguy et al. (3) obtained remissions in DMBA tumor-bearing rats treated with either 10 or 25 μg of A-43818 twice daily for 6 weeks. Both doses produced tumor remissions, although, contrary to the report by DeSombre et al. (4), the drug was less effective than ovariectomy. Plasma prolactin levels were reduced by approximately 50% in the animals treated with the 10-μg doses, but, despite the occurrence of tumor remissions, there was no significant change in those receiving 25 μg. Ovarian estrogen secretion...
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was considered to be unaffected because estrus cycles were maintained. In fact, however, they did note that the estrus cycles were prolonged and irregular in their A-43818-treated animals. Their overall conclusion was that A-43818 exerts its antitumor effect by a specific prolactin-inhibiting activity, either directly at the level of the pituitary or by modifying the hypothalamic regulatory mechanism.

Both estrogens and prolactin are known to influence the growth of DMBA-induced rat mammary carcinomas, although the dominant hormone appears to be prolactin (8). Ovariectomy, like A-43818, induces remission of these tumors and results in reduced circulating prolactin, as well as estrogen, levels. Estrogen replacement after ovariectomy causes a significant increase in plasma prolactin (1). Sterental et al. (11) found that, after remissions have been induced by ovariectomy and adrenalectomy, daily treatment with 5 μg of estradiol benzoate reactivates tumor growth; no such effect occurs after hypophysectomy-induced remissions.

The study reported here shows that the tumor-suppressive action of A-43818 is also prevented by estradiol benzoate administration. In addition, perphenazine, which causes an increase in plasma prolactin and consequent reactivation of DMBA-induced mammary tumor growth after ovariectomy (8, 9), was found to have a similar action in A-43818-treated animals. These results are consistent with A-43818’s exerting its antitumor effect by inhibition of ovarian steroidogenesis with secondary hypoprolactinemia. Plasma prolactin levels were not assayed, but it seems most likely that the simultaneous administration of either estradiol benzoate or perphenazine reactivated tumor growth by increasing the circulating level of this hormone.

In another study (10), we investigated the activity of A-43818 against rat mammary carcinomas induced by N-nitrosomethyurea. These tumors regress after treatment with the analog and also respond to ovariectomy and the antiestrogen tamoxifen. However, unlike DMBA-induced tumors the efficacy of A-43818 is not influenced by perphenazine, although it is inhibited by estradiol benzoate. Thus, these carcinomas appear to be estrogen dependent rather than prolactin dependent, the analog acting directly by producing a chemical ovariectomy. In this respect, N-nitrosomethyurea-induced mammary cancers more closely model the human disease in which antiprolactin agents have failed to produce objective remissions (5).

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REFERENCES

Modification of the Effect of a Gonadoliberin Analog on 7,12-Dimethylbenz(a)anthracene-induced Rat Mammary Tumors by Hormone Replacement

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