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Effect of Catechol Estrogens on Rat Mammary Tumors[1]

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ABSTRACT

The effect of 1,3,5(10)-estratriene-3,16α,17β-triol (estriol), 1,3,5(10)-estratriene-2,3-diol-17-one (2-hydroxyestrone), and 1,3,5(10)-estratriene-2,3,17β-triol (2-hydroxyestradiol) on the growth of dimethylbenz(a)anthracene-induced mammary tumor and of R3230AC-transplantable mammary tumor was compared with that produced by estradiol benzoate treatment. Estriol showed minimal inhibition of tumor growth in dimethylbenz(a)anthracene-induced tumor and no effect on R3230AC tumor while 2-hydroxyestrone showed no effect of tumor inhibition. On the other hand, 2-hydroxyestradiol showed appreciable inhibition of tumor growth in both tumors studied. That 2-hydroxyestradiol has been found to bind to estrogen receptors in mammary tumors and is uterotropic suggests that the inhibition of tumor growth by 2-hydroxyestradiol may be similar to the mechanism of inhibition of mammary tumors by high concentrations of estradiol.

INTRODUCTION

One of the postulated reasons for the lower incidence of breast cancer in Japan, compared with that in most Western countries (9, 27), is believed to be differences in estrogen levels between the 2 populations. In support of this hypothesis, several workers have shown that the "estriol[2] ratio" [estriol/(estrone plus estradiol)] is higher in Japanese women than in Caucasians (5, 18) and is also higher in low-risk populations (5, 22). Furthermore, support for this hypothesis was based on the following observations: (*a*) estriol has minimal estrogenic effects and can act as an antagonist to the uterotropic effect of estradiol (14); (*b*) estriol does not produce mammary cancer in rats in contrast to estradiol and estrone (11); (*c*) low incidence of DMBA-induced tumors in rats given estriol before DMBA administration as compared with rats given DMBA alone (16); and (*d*) estriol lowers the binding of estradiol to its cytoplasmic receptor (17, 30). However, there are several lines of evidence that do not support the estriol hypothesis. The first is that estriol has been found now to be carcinogenic in mice (26), and the second is based on results obtained by Anderson *et al*. (2) and Clark *et al*. (6) who showed that estriol is in fact an estradiol agonist when administered chronically and has the same uterotropic activity as estradiol. Furthermore, Deshpande *et al*. (8) showed no evidence that estriol inhibited the uptake of tritiated estradiol in the tumors of patients with breast cancer. Finally, results obtained by Pratt and Longcope indicate no significant differences in estriol production rates in women with previous breast cancer *versus* normal women (25). These new results seem to suggest that the "estriol hypothesis" as initially conceived is no longer valid in explaining altered breast cancer risk in certain women or in defined populations.

Conversely, there is new evidence that the catechol estrogens, 2-hydroxyestrone and 2-hydroxyestradiol, may play the antiestrogen role that has been ascribed to estriol. Reports on catechol estrogens show these to be the major metabolites in humans and animals (3, 4). These metabolites compete with estradiol for cytosol estrogen receptors of the hypothalamus and pituitary (7) suggesting a role for these compounds in the control of gonadotropin secretion within the pituitary-hypothalamic axis (10, 23, 24). On the other hand, 2-hydroxyestradiol, which competes for estrogen receptors in rat uterine tissue (19) and in DMBA-induced rat mammary tumor tissue (1) as well as causes significant nuclear receptor translocation (1), has been shown to have appreciable uterotropic activity (1, 20).

Since 2-hydroxyestradiol can act as both an estrogen and antiestrogen, we carried out this investigation to study the effects of catechol estrogens on tumor growth responses in DMBA-induced and R3230AC-transplanted rat mammary carcinomas and to compare them with tumor growth responses obtained using estradiol and estriol.

MATERIALS AND METHODS

Steroids. Estradiol benzoate and estriol were obtained from Sigma Chemical Co., St. Louis, Mo. Catechol estrogens were synthesized according to the procedure of Stubenrauch and Knuppen (28). The purified compounds were recrystallized and shown to be homogeneous by thin-layer chromatography. The absence of trace amounts of estradiol and estrone in the purified samples was established by contaminating the solution with [^{3}H]estrone and estradiol, and the mixture was chromatographed on thin-layer chromatography. Recrystallization from methanol gave pure 2-hydroxyestradiol and 2-hydroxyestrone devoid of radioactivity.

DMBA Tumor Induction, Growth, and Treatment. Fifty-five-day-old female Sprague-Dawley rats (obtained from BioLabs, St. Paul, Minn.) were given 20 mg of DMBA in 1 ml of sesame oil by gastric intubation. Eighty % of the rats developed breast tumors between 5 weeks and 4 months after treatment with DMBA. Rats were palpated for tumors at weekly intervals, the size was recorded as the mean of 2 perpendicular diameters, one measured across the greatest width, and tumor surface area was plotted on a growth chart. Since it was desired that each treatment group contain about the same tumor size at the initiation of therapy, animals were placed in a treatment group after their tumors had reached the desired size range (from 6 to 8 sq cm). The average time after appearance of the tumor to initiation of therapy was 3 weeks. Treatment of all groups was continued for an additional 5 weeks. The animals were divided

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[2] The abbreviations used are: estriol, 1,3,5(10)-estratriene-3,16α,17β-triol; DMBA, 7,12-dimethylbenz(a)anthracene; 2-hydroxyestrone, 1,3,5(10)-estratriene-2,3-diol-17-one; 2-hydroxyestradiol, 1,3,5(10)-estratriene-2,3,17β-triol; estradiol benzoate, 1,3,5(10)-estratriene-3-17β-diol-3-benzoate.

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into 6 treatment groups with 10 animals in each group. The following daily injections were given: Group A, 0.1 ml of corn oil (controls); Group B, 20 μg of estradiol benzoate; Group C, 100 μg of estriol; Group D, 100 μg of 2-hydroxyestradiol; Group E, 100 μg of 2-hydroxyestrone; and Group F, 20 μg of estradiol benzoate plus 100 μg of 2-hydroxyestradiol. All injections were given s.c. Mammary carcinomas were considered to be regressing if the tumor size decreased by at least 30% from the beginning to end of therapy and advancing if the tumor size increased (12).

R3230AC-transplanted Tumor and Treatment. Female Fischer 344 rats weighing 120 to 140 g received tumor transplants by sterile trochar technique. The animals were divided into 6 treatment groups with 10 animals in each group. Daily injections of vehicle and test compounds were given s.c. on Day 1 following transplantation and continued for a total of 21 days when the experiment was terminated. Injections given were: Group G, 0.1 ml of corn oil (controls); Group H, 20 μg of estradiol benzoate; Group I, 100 μg of estriol; Group J, 100 μg of 2-hydroxyestradiol; Group K, 100 μg of 2-hydroxyestrone; and Group L, 20 μg of estradiol benzoate plus 100 μg of 2-hydroxyestradiol. Rat weights were recorded on Days 1, 10, and 18, and on Day 21, all animals were killed and necropsies performed.

RESULTS

The effects of treatment with estradiol, estriol, 2-hydroxyestradiol, and 2-hydroxyestrone on the growth of DMBA-induced rat mammary carcinomas are shown in Charts 1 to 4 and Table 1. The tumors of control animals grew exponentially. Of the estradiol-treated group, 8 regressed and 2 advanced in growth similar to the control curve (Chart 1; Table 1). 2-Hydroxyestradiol treatment appeared to be about as therapeutically efficient as estradiol in that 6 tumors of 2-hydroxyestradiol-treated animals regressed while 4 continued to grow (Chart 2; Table 1). However, the number of rats with no evidence of tumors at the end of the treatment period is 5 for the estradiol-treated group and only 2 for the 2-hydroxyestradiol-treated group. Treatment with 2-hydroxyestrone (Chart 3; Table 1) showed no tumor inhibition. Treatment with estriol (Chart 4; Table 1) resulted in regression in only 3 tumors while the remaining 7 tumors advanced in growth similar to that in the control group. Furthermore, all of the regressed tumors showed the presence of tumor growth at the end of the treatment period (Table 1). When both estradiol and 2-hydroxyestradiol were administered simultaneously, results similar to those obtained by estradiol administration only were observed (Chart 5; Table 1).

Table 2 shows the results obtained from treatment with estradiol, estriol, 2-hydroxyestrone, 2-hydroxyestradiol, and estradiol plus 2-hydroxyestradiol on the growth of R3230AC-transplantable rat mammary carcinoma. A significant reduction of tumor growth was observed at a 20-μg/day dose of estradiol

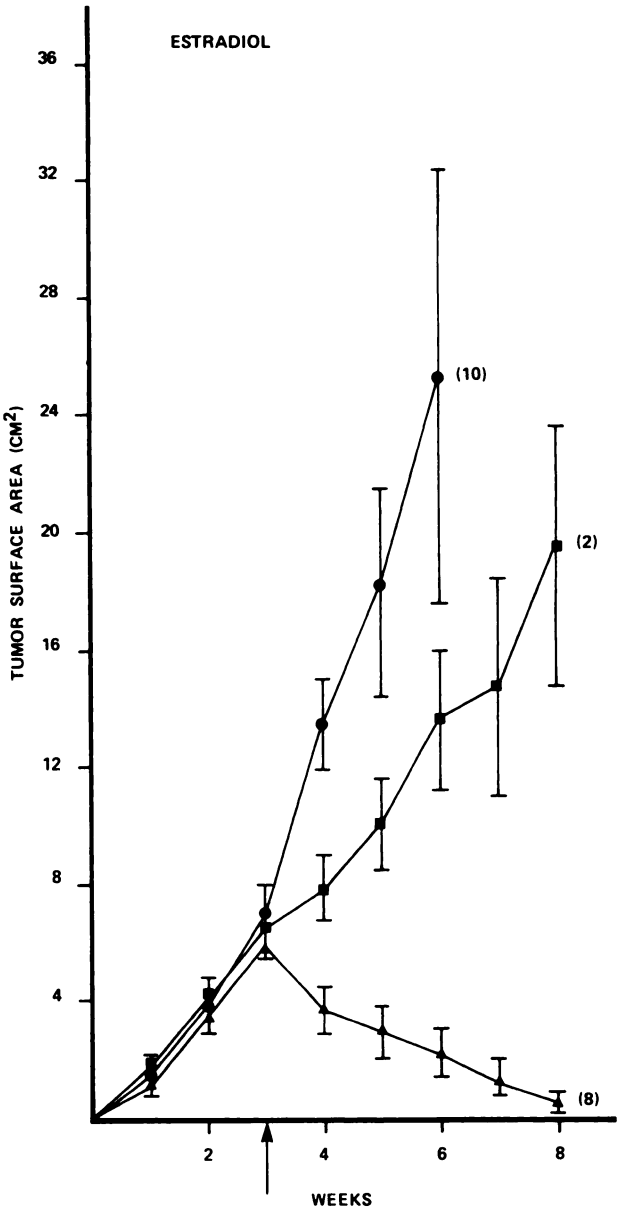

Chart 1. Effect of estradiol benzoate administration on DMBA tumor growth. Average weekly surface area of 10 control tumors (●), 8 regressing tumors (▲), and 2 advancing tumors (■). *Bars*, S.E.; *Arrow*, time when treatment was begun.

Table 1

Effects of treatment in vivo of DMBA-induced rat mammary carcinoma with estrogens

Group	Treatment	Dose (μg/day)	Total no.	Regressing carcinomas (>30% size decrease)	Advancing carcinomas	No evidence of carcinoma at end of treatment period
A	Control		10	0	10	0
B	Estradiol benzoate	20	10	8	2	5
C	Estriol	100	10	3	7	0
D	2-Hydroxyestradiol	100	10	6	4	2
E	2-Hydroxyestrone	100	10	0	10	0
F	Estradiol benzoate plus 2-hydroxyestradiol	20 100	10	9	1	6

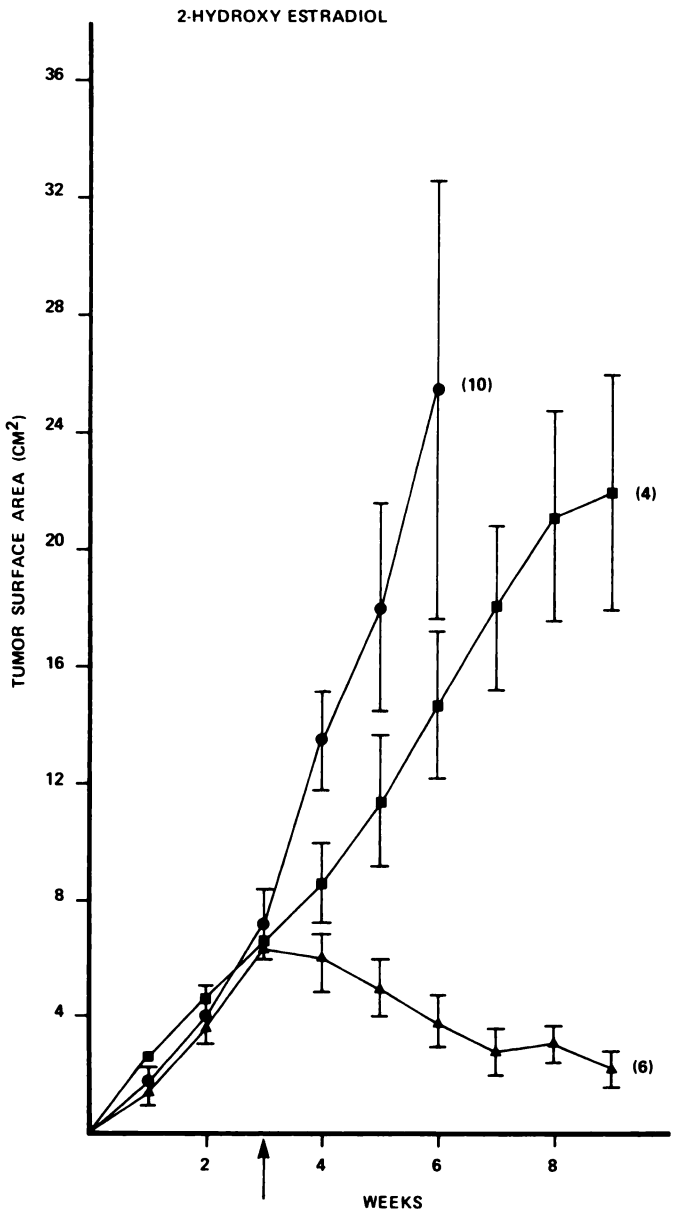

Chart 2. Effect of 2-hydroxyestradiol administration on DMBA tumor growth. Average weekly surface area of 10 control tumors (●), 6 regressing tumors (▲), and 4 advancing tumors (■). *Bars*, S.E. *Arrow*, time when treatment was begun.

benzoate. The ratio of treated to control tumor weights is shown in Table 2, Column 5. Treatment with 2-hydroxyestradiol resulted in significant inhibition of tumor growth within 21 days. However, estriol and 2-hydroxyestrone administration showed very little tumor-inhibitory activity. Furthermore, the treated/control ratio obtained from administration of estradiol plus 2-hydroxyestradiol was essentially similar to that obtained by estradiol administration.

DISCUSSION

In the present study, we have presented data on the effectiveness of estradiol, 2-hydroxyestradiol, 2-hydroxyestrone, and estriol on the growth of DMBA and R3230AC tumors. High-dose estradiol benzoate administration has been found to inhibit tumor growth in both of these mammary tumors. These results are essentially similar to those obtained by other investigators on DMBA (15, 21) and R3230AC (13, 29) tumor regression following estradiol administration. In sharp contrast to these observations is the effect of estriol on the growth of mammary tumors. As can be seen from Chart 4 and Table 1, estriol administration resulted in a significant regression in only 30% of DMBA tumors with all regressing tumors showing some tumor growth at the end of the treatment period. These results are in variance with the results obtained by Lemon (16) who showed a lower incidence of DMBA-induced tumors in rats given estriol before DMBA administration as compared with rats given DMBA alone (16). However, these workers were studying the protective effects of estriol on the induction of tumors by DMBA as compared to our studies on tumor inhibition by estriol, and it is quite conceivable that different mechanisms are involved using these 2 different experimental designs. Thus, the results obtained in our study of DMBA indicate that estriol has little antitumor activity if any at all. Further support for these results is obtained from our studies on the effect of estriol on the growth of the hormone-responsive autonomous R3230AC carcinoma. Table 2 shows that estriol had no antitumor activity as observed from the treated/control ratio of

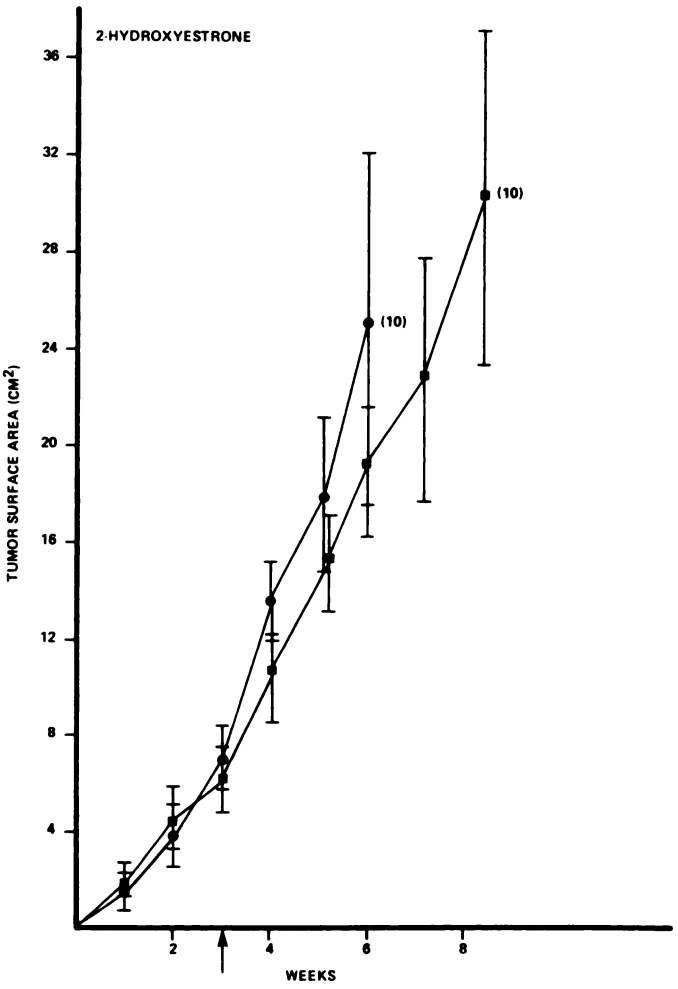

Chart 3. Effect of 2-hydroxyestrone administration on DMBA tumor growth. Average weekly surface area of control tumors (●) and 10 advancing tumors (■). *Bars*, S.E. *Arrow*, time when treatment was begun.

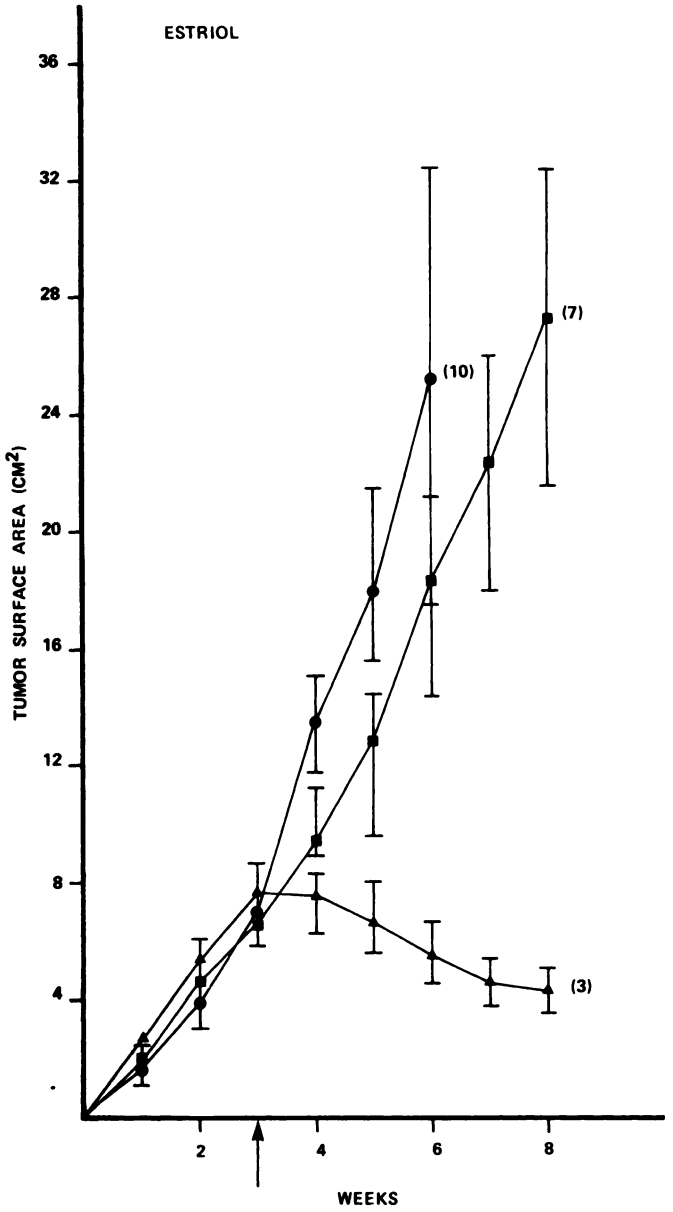

Chart 4. Effect of estriol administration on DMBA tumor growth. Average weekly surface area of 10 control tumors (●), 3 regressing tumors (▲), and 7 advancing tumors (■). *Bars*, S.E. *Arrow*, time when treatment was begun.

0.94. Our results suggest that estriol is not antiestrogenic and support other results which show that estriol is in fact a potent estrogen as determined by its uterotropic activity (6, 19), binding to estradiol receptor, induction of tumors in mice (26), and inability to inhibit tumor growth.

In view of the controversy over the role of estriol in human breast cancer (31) and the recent results obtained on the antiestrogenic (10, 23, 24) and estrogenic activities (1, 20) of the catechol estrogens, we attempted to study the antitumor activity of 2-hydroxyestradiol and 2-hydroxyestrone in rat mammary tumors. As seen in Chart 2 and Table 1, 2-hydroxyestradiol shows a significant inhibitory effect on the growth of DMBA mammary tumors. Sixty % of the tumors regress with 2 of 6 tumors showing no sign of tumor growth at the end of the treatment period. Similarly, administration of 2-hydroxyestradiol to Fischer rats bearing R3230AC tumors showed remarkable inhibition of tumor growth with a treated/control ratio of 0.70 as shown in Table 2. That 2-hydroxyestradiol binds to cytosol estrogen receptors from mammary tumors and is capable of inducing translocation of receptor (1) as well as having appreciable uterotropic activity (1, 20) suggests that 2-hydroxyestradiol may act as an estrogen agonist. Furthermore, results obtained from experiments where both estradiol and 2-hydroxyestradiol are administered simultaneously (Chart 5; Tables 1 and 2) show that 2-hydroxyestradiol has no estrogen antago-

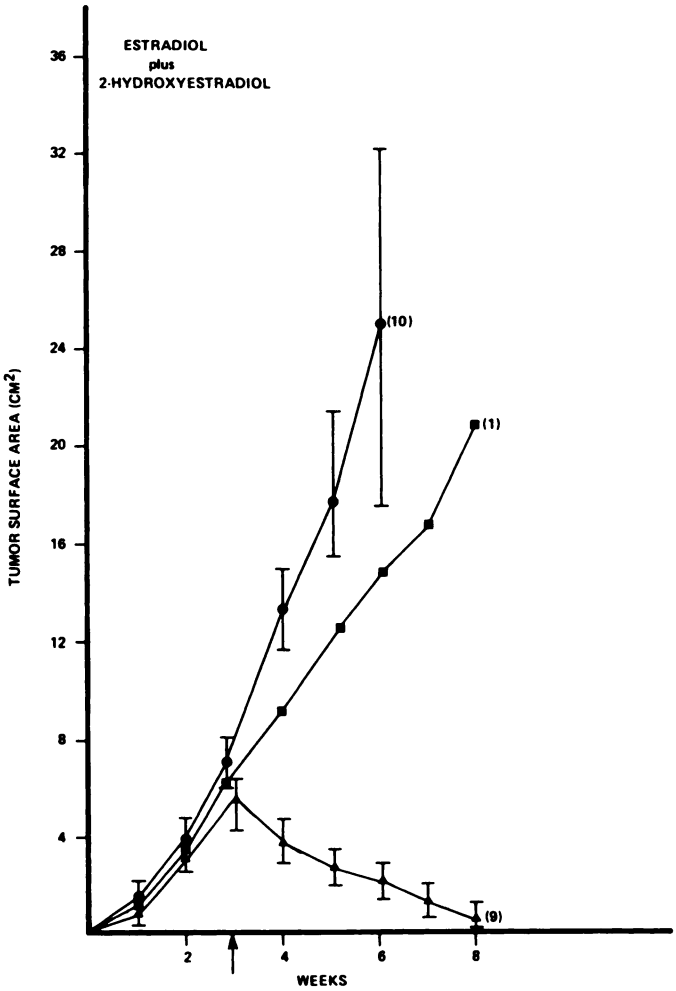

Chart 5. Effect of estradiol benzoate plus 2-hydroxyestradiol administration on DMBA tumor growth. Average weekly surface area of 10 control tumors (●), 9 regressing tumors (▲), and one advancing tumor (■). *Bars*, S.E. *Arrow*, time when treatment was begun.

Table 2

Effects of treatment in vivo of R3230AC-transplantable rat mammary carcinoma with estrogens

Group	Treatment	Dose (μg/day)	Tumor wt (mg)[a]	T/C[b]
G	Vehicle		1.74 ± 0.26	
H	Estradiol benzoate	20	0.98 ± 0.13	0.56
I	Estriol	100	1.63 ± 0.19	0.94
J	2-Hydroxyestradiol	100	1.21 ± 0.09	0.70
K	2-Hydroxyestrone	100	1.76 ± 0.20	1.01
L	Estradiol benzoate plus 2-hydroxyestradiol	20 100	0.87 ± 0.18	0.50

[a] Mean ± S.E. in that group as recorded on Day 21.
[b] Weight of tumor in treated group/weight of tumor in control group.

nistic activity and are in agreement with earlier studies on receptor binding and estrogenic response (1). However, unlike 2-hydroxyestradiol, that 2-hydroxyestrone showed no inhibition in the two mammary tumors investigated supports earlier results which show that 2-hydroxyestrone cannot induce receptor translocation (1) and has no estrogenic (1, 20) or antiestrogenic activity (20).

The results obtained from this study show that 2-hydroxyestradiol, which has uterotropic activity and is capable of inducing receptor translocation, can inhibit tumor growth. Furthermore, estriol, which is known to be uterotropic and also binds to estrogen receptors, shows minimal tumor regression. That full estrogenic response requires chronic administration of estriol (6) may suggest that the weak antitumor activity of estriol observed in these studies results from the insufficiently frequent administration of this short-lived estrogen agonist. These studies lead us to conclude that these compounds are estrogenic and that the antitumor activity observed in this study for estriol and 2-hydroxyestradiol is very probably due to a mode of action similar to the mechanism of inhibition of human breast cancer by high concentrations of estradiol.

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