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Increased Survival Due to Radioactive Estradiol in Mice with C3HBA or BW 10232 Tumors

Benjamin Thysen[1,4,5], Giuseppe Rettura[2], Jacques Padawer[3], Michael Gatz[1], Stanley M. Levenson[2], and Eli Seifter[2,4]

[1] Department of Laboratory Medicine, [2] Department of Surgery, [3] Department of Anatomy, [4] Department of Biochemistry, and [5] Department of Gynecology and Obstetrics, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, USA

Summary. *The influence of progesterone and estradiol labeled with tritium was studied in mice inoculated with transplantable mammary adenocarcinomas C3HBA or BW 10232. Tumor size, tumor growth rate, and host survival were measured. Radioactive [^{3}H]estradiol administration increased survival time and inhibited tumor growth in mice inoculated with these tumor lines. Tumor growth retardation depended on the amount of radioactivity injected and nonradioactive estradiol was without any salutary effect on tumor size or host survival. Neither survival times nor tumor growth rate were altered by radioactive [^{3}H]progesterone. The underlying mechanism(s) is (are) referable to ionizing radiation by the specific carrier estradiol or to an isotope effect of [^{3}H]estradiol.*

Introduction

Administration of an analog of gonadotropin-releasing hormone (GnRH) has been reported to induce regression of a spontaneous rat mammary neoplasm and of tumors which had been induced with dimethylbenzanthracene [5]. Administration of a luteinizing hormone-releasing hormone (LHRH) agonist has been shown to cause regression of estrogen receptor-positive mammary tumors as well as regression of some estrogen receptor-negative tumors [8, 9]. The role of GnRH in such hitherto described hormone-independent tumors merits further investigation.

Because estradiol is a factor that moderates LHRH release [11, 14] in a dose-dependent way, we hypothesized that administration of estradiol modified in the form of radioactive estradiol to animals bearing steroid-independent neoplasms might cause a change in the naturally occurring LHRH output, and thereby affect tumor growth. These considerations led us to test the hypothesis that administered [^{3}H]estradiol would inhibit the growth of transplanted mammary tumors. The purpose of this investigation was to study the effect of graded doses of labeled and unlabeled estradiol (when given in doses comparable to those used in physiologic and pharmacologic experiments) on tumor growth in mice and host survival.

Reprint requests should be addressed to B. Thysen

Materials and Methods

C3HBA and BW 10232 tumors are spontaneous mouse mammary adenocarcinomas [4, 7, 12, 13]. The tumors were obtained from the Jackson Laboratories (Bar Harbor, ME) and maintained in young female C3H/HeJ mice and C57BL/6J mice, respectively, by transfer of viable cells. For the present study, transplants in female mice were prepared as follows:

Tumors from five mice were excised under sterile conditions and washed with Hank's buffered salt solution. The tissue was gently minced, filtered through a stainless steel no. 100 mesh screen, and centrifuged at 1800 *g* for 10 min, after which the cells were resuspended in Dulbecco's phosphate-buffered saline at pH 7.2 (Grand Island Biological Company, Grand Island, NY). Groups of 10 or 15 mice (6 weeks old) each received SC inocula in the inguinal region of 1 million cells (for the C3HBA tumor) or 200,000 cells (for the BW 10232 tumor) in 0.1 ml phosphate buffer. The mice were housed in plastic shoebox-type cages (five mice per cage) made of opaque polyethylene with a three mesh galvanized wire top, measuring 5 in. × 8 in. × 12 in., and kept in an artificially lighted room (light 07:00 to 19:00) at a constant temperature of 23° C. The mice were fed commercial chow (Teklad) and received water ad libitum. The animals were examined daily by gentle palpation to determine the day of tumor appearance. Thereafter, survival time was measured. Hormone injections were given SC in the cervical region to each animal on the side opposite the inoculum 5–7 days following tumor inoculation. Peanut oil, the vehicle, was injected into control mice.

The radioactive 17B-[2,4,6,7-^{3}H]estradiol and the [1,2,6,7-^{3}H]progesterone had a specific activity of 90 Ci/mmol (New England Nuclear Corporation, Boston, MA, USA).

The radiochemical purity of estradiol was verified by paper chromatography on Whatman no. 1 with benzene : hexane : meth-

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anol : water (33 : 66 : 80 : 20). The radiochemical purity of progesterone was verified by thin-layer chromatography in cyclohexane : ethyl acetate (1 : 1). The purity of each was found to be > 98%.

The data expressed as means ± SE were analyzed by analysis of variance (ANOVA) [16]. Where warranted by the F statistic, they were further examined by the Newman Keuls tests to establish allowable comparisons and the P values for these comparisons [17].

Estimation of Tumor Growth. In the first experiment, tumor growth was approximated with a millimeter rule. Values were assigned as follows: 1+ = 1–3 mm; 2+ = 4–6 mm; 3+ = 7–9 mm; 4+ = 10–13 mm; 5+ = 14–16 mm.

In all subsequent experiments, tumor growth was measured with calipers to the nearest millimeter, three times a week. The long and short perpendicular diameters of each tumor were averaged to express a mean tumor size. Values expressed as a fraction of a millimeter, in Tables 2–5, were obtained from derived data. The tumor measurements reported in this study were determined at various days following tumor inoculation.

Results

The effects of 1–50 μCi tritiated estradiol on tumor growth and host survival in the C3HBA model are shown in Table 1. Treatment for 4 days with tetralabeled tritiated estradiol resulted in about 50% increase in mean survival time. In the dose range studied, host survival was not dose-dependent beyond 1 μCi, since the survival time achieved with 5 or 50 μCi was no better than that obtained with 1 μCi.

The mean host survival of animals given 1,150 ng unlabeled estradiol plus 50 μCi radioactive estradiol did not differ significantly from vehicle controls, suggesting that unlabeled estradiol can inhibit the action of [^{3}H]estradiol. Tumor growth inhibition appeared to be related to increased survival, but the accuracy of tumor measurements did not justify analysis by statistical means in this first experiment.

In contrast to the response with radioactive estradiol, no effect on host survival time was found when comparable amounts of nonradioactive estradiol were administered for 4 days (Table 2). Similarly, no measurable effect on tumor size was seen with these treatments.

The influence of further, prolonged [2,4,6,7-^{3}H]estradiol treatments, and treatments with lower dosages without change in specific activity is shown in Table 3. Radioactive estradiol administration for 14 days at dosages of 0.2–2.0 μCi/day resulted not only in a significant increase in animal survival time, but also in a significant inhibition of tumor growth. There is a dose-dependent response for growth retardation in the range 0.1–1.0 μCi/day. While mean survival was increased in this experiment, with daily injections of 0.2–2.0 μCi tritiated estradiol the survival times (approx. 60 days) were similar over the entire dosage range. With the 0.1 μCi treatment, neither tumor growth nor survival time differed significantly from the controls.

The response of 0.2 μCi injections of radioactive progesterone or radioactive estradiol per day on tumor growth in C57BL/6J female mice inoculated with BW 10232 tumor cells is shown in Table 4. Considerable variation in tumor size was found between animals in each group. When the differences in size were used (e.g., between days 7 and 9 and between days 7 and 15) statistical significance was indicated (Table 5). Tumor growth rate was inhibited by 85% after only 2 days' treatment with [^{3}H]estradiol. After 8 days' treatment, the inhibitory effect on tumor growth rate declined to 36% and remained at this level until 12 days post hormone injection (Table 4). Furthermore, as a result of radioactive estradiol administration for 14 days, survival time in these tumor bearing mice was enhanced by 75% (Table 5).

Table 1. Effect of [^{3}H]estradiol in 7-week-old C3H/HeJ female mice bearing C3HBA tumor

Group[a]	^{3}H-Estradiol[b]		Survival time[c] (days)	Tumor size[g]		
	μCi	ng		Day 7	Day 12	Day 20
Control	0	0	41.2 ± 3.7	2+	3+	5+
A	1	2.6	60.9 ± 2.4[d]	1+	2+	3+
B	5	13	56.1 ± 2.6[e]	2+	2+	5+
C	50	130	60.8 ± 2.4[d]	1+	2+	3+
D	50	1,150	48.2 ± 1.9[f]	2+	2+	5+

[a] Ten animals per group
[b] μCi/mouse/day
[c] Mean ± SE
[d] Comparison with control, $P < 0.001$
[e] Comparison with control, $P < 0.01$
[f] Comparison with control, $P > 0.05$
[g] Qualitative assessment made from 1+ to 5+. See explanation in *Materials and Method*

Table 2. Effect of nonradioactive estradiol in 7-week-old C3H/HeJ female mice bearing C3HBA tumor

Group[a]	Nonlabeled[b] estradiol (ng)	Survival time[c, d] (days)	Tumor size[c, d] (mm)		
			Day 7	Day 10	Day 15
Control	0	43.0±1.6	4.3±0.3	9.4±0.3	18.4±0.4
A	0.5	44.0±0.8	4.2±0.4	9.5±0.5	18.0±0.5
B	2.6	41.6±1.5	3.9±0.5	9.2±0.4	17.9±0.6
C	13.0	44.0±0.6	3.8±0.2	9.0±0.5	17.6±0.6
D	1,150	41.8±1.3	3.9±0.4	9.8±0.5	18.3±0.3

[a] Ten animals per group
[b] ng/mouse/day
[c] Mean ± SE
[d] Comparison with control, $P > 0.05$

Table 3. Effect of [^{3}H]estradiol in 7-week-old C3H/HeJ female mice bearing C3HBA tumor

Group[a]	[^{3}H]Estradiol		Survival time[b] (days)	Tumor size (mm)		
	μCi	ng		Day 12	Day 20	Day 27
Control	0	0	41.8 ± 1.2	8.6 ± 0.5	19.1 ± 0.3	24.0 ± 0.6
A	0.1	0.26	43.2 ± 1.8[c]	6.1 ± 0.4[e]	16.3 ± 1.0[c]	21.8 ± 1.1[c]
B	0.2	0.52	60.1 ± 1.0[d]	4.6 ± 0.5[d]	13.4 ± 0.6[d]	18.6 ± 0.6[d]
C	1.0	2.6	57.2 ± 2.4[d]	4.1 ± 0.4[d]	12.4 ± 0.9[d]	16.6 ± 0.9[d]
D	2.0	5.2	63.8 ± 3.4[d]	5.3 ± 0.3[d]	11.6 ± 0.7[d]	17.1 ± 0.7[d]

[a] Fifteen animals per group
[b] Mean ± SE
[c] Comparison with control, $P > 0.05$
[d] Comparison with control, $P < 0.001$
[e] Comparison with control, $P < 0.01$

Table 4. Effect of [^{3}H]estradiol and [^{3}H]progesterone on tumor growth in animals bearing BW 10232 tumor

		Tumor score (mm)								
Day post inoculum		7	8	9	12	13	14	15	16	19
Day post hormone injection		0	1	2	5	6	7	8	9	12
Control	Mean	1.8	2.6	3.8	6.6	9.1	11.9	13.7	17.4	19.9
	Standard error	±0.3	±0.4	±0.4	±0.7	± 0.8	± 1.1	± 0.8	± 0.9	± 0.8
[^{3}H]-Estradiol	Mean	2.0	2.2	2.3	4.8	6.7	8.3	9.7	11.5	14.3
	Standard error	±0.3	±0.4	±0.4	±0.7	± 1.0	± 1.0	± 0.9	± 1.1	± 1.2
[^{3}H]-Progesterone	Mean	1.8	2.8	3.6	7.5	10.0	12.0	14.1	17.0	19.9
	Standard error	±0.3	±0.5	±0.5	±1.1	± 1.3	± 1.5	± 1.4	± 1.5	± 1.3

[a] Animal died

Table 5. Effect of radioactive hormones on survival time and the change in tumor size in 6-week-old C57BL/6J female mice bearing BW 10232 tumor

Group[a]	Survival time (days)	Δ Tumor size[e] (mm)	
		Day 9−day 7	Day 15−day 7
Control	20.3 ± 0.4	2.0 ± 0.2	11.9 ± 1.1
[^{3}H] Estradiol	35.1 ± 1.9[b]	0.3 ± 0.2[b]	7.7 ± 0.3[f]
[^{3}H] Progesterone	22.3 ± 0.4[c, d]	1.8 ± 0.3[c, d]	12.3 ± 1.7[c, d]

[a] Ten animals per group
[b] Comparison with control, $P < 0.001$
[c] Comparison with control, $P > 0.05$
[d] Comparison with estradiol, $P < 0.005$
[e] Mean ± SE
[f] Comparison with control, $P < 0.005$

In contrast, radioactive progesterone had no effect on these measurable parameters.

Discussion

In the present study, 4-day treatment of radioactive estradiol, in the form of tritium-labeled estradiol at positions 2, 4, 6, and 7 has been found to increase survival in two different strains of mice bearing two different autonomous mammary adenocarcinomas. Increasing treatment to 14 days did not enhance host survival above that achieved with the 4-day radioactive estradiol treatment, but did result in retardation of the C3HBA tumor size. The inhibitory effect on growth of this breast neoplasm lasted for 27 days following tumor inoculation, and appeared to be related to the amount of radioactive estradiol administered. These findings indicated that further treatment with radioactive estradiol injections over a longer period of time may extend host survival as well as tumor growth inhibition.

The present studies were an outgrowth of an hypothesis (E.S.) that radioactive estrogens would exert radiolytic action in breast tumors. Estrogen receptors have been reported in low amounts in mammary carcinomas of C3H mice [2] and virtually none could be detected by Padawer et al. [10]. We have reported that C3HBA tumor can grow in both male and female C3H/HeJ mice [15]. Clearly, a mechanism other than radiolysis of estrogen receptors in the neoplasm was in operation. Since nonradioactive estradiol was without effect (Table 2), we considered the possibility that the hormone, modified in the form of tritium-labeled estradiol might be implicated in the regulation of mouse host survival and tumor growth.

In an attempt to examine isotope carriers other than estradiol, radioactive progesterone was given to mice bearing BW 10232 mammary adenocarcinomas. No change in tumor growth rate or survival time was noted as a consequence of 0.2 μCi [^{3}H]progesterone treatments for 14 days. However, daily administration of radioactive estradiol beginning at 8 days post inoculum evoked an inhibition of tumor growth rate until 19 days post inoculum and enhanced survival time in these animals. The difference in the activities of the two tritiated steroids may be related to difference in action at specific site(s). Accordingly, the antitumor activity was not indiscriminate, but was associated with specific carrier molecules.

Recently, analogues of LHRH have been found to be effective in the regression of DMBA-induced tumors in rats [5, 8, 9], in a spontaneous rat mammary adenocarcinoma [5], and in some estrogen receptor-negative rat mammary tumors [8, 9]. Modulation of rat LHRH release appears to be controlled by estrogen receptors in the median basal hypothalamus [3, 6]. It is possible that the antitumor and enhanced host survival effects in our study may have been due to an increased secretion of LHRH, as a consequence of the action of radioactive estradiol at these specific hypothalamic receptors. Although we present no data in the present report, it is unlikely that ionizing radiation was involved, since significant tumor growth rate inhibition in the C57BL/6J mice had been noted after only 2 days' treatment with only 0.2 μCi tritiated estradiol per day. However, the effect of exposing hypothalamic cells to a radionuclide on neurosecretion is unknown. Indeed, central nervous system defects of various types can be produced by radiation in experimental animals [1].

We speculate that [^{3}H]estradiol, due to isotope effects, may have acted as a weak estradiol agonist at the hypothalamus. Experiments are currently being conducted to define the means by which radioactive estradiol alters tumor growth and mouse host survival.

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