Inhibition of Radiogenic Mammary Carcinoma in Rats by Estriol or Tamoxifen

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Mammary carcinomas have been induced by 3.5 Gy whole-body gamma radiation administered at age 40 to 50 days to virgin female Sprague-Dawley rats. In 142 irradiated controls carcinoma incidence averaged 7.8% in survivors observed less than 300 days and 38.3% of those surviving longer ($P < 0.001$ by t test). Mammary cancer promotion was inhibited by two methods: estriol (E3) 638 μg/month (2.2 μm/mo) subcutaneously for natural life span begun 2 weeks after exposure reduced cancer incidence from 76% in controls to 48% after 331 to 449 mean days observation until neoplasia was palpable ($P < 0.02$ by chi-square analysis). Uterine weights were similar in control and treated groups, and were 15% to 18% greater than uteri of nonirradiated controls from other simultaneous experiments. Six monthly 638-μg doses of 17 alpha ethinyl estriol (EE3) reduced tumors from 88% in controls to 64% ($P < 0.05$ by chi-square analysis) and delayed cancer onset ($P < 0.01$–0.04 by life table analysis). Ethinyl estradiol (EE2) after 6 months' treatment similarly delayed mammary tumor development reducing incidence to 75% (NS), with a six-fold increase in nonmammary epithelial malignant tumors. Estriol administration begun between 3 days before to 5 days after radiation did not alter mammary cancer incidence in six experiments. Monthly implantation of 2.5 mg tamoxifen (4.44 μm/mo) started 2 weeks after radiation reduced mammary cancer incidence from 83% to 14% after 307 to 314 days' observation ($P < 0.001$ by chi-square analysis). Treated rats had atrophic ovaries and uteri consistent with blockade of endogenous estradiol activity. Short-term parenteral E3 or EE3 therapy using 10 to 30 μg/kg/day (35–100 μm/kg/day) rapidly differentiated virgin rat mammary glands without impairment of subsequent estrus cycles and offers an alternative to castration or life-long antiestrogen therapy for reduction of risk of radiogenic mammary carcinoma.

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RADIATION-INDUCED mammary neoplasia has been seldom investigated concerning potential methods of prophylaxis after exposure, unlike chemically induced neoplasia. Neutron and gamma irradiation induce mammary carcinoma in women and in female rats,[1-10] in whom a linear dependence of tumor incidence upon the absorbed radiation dose has been found.[11] New clinical cases of breast cancer continue to be reported 10 to 15 years or longer after exposure to repeated chest or cardiac fluoroscopy, thymic irradiation, and mammary irradiation for benign disease or incidental to therapeutic irradiation of mediastinal tumors.[1-7]

Thus far the contralateral breast does not seem to be at risk for well-planned radiation directed at controlling neoplasia on the opposite side.[8] Many young women have now received up to 12.0 Gy whole-body fractional irradiation in preparation for allogeneic or autologous bone marrow transplantation for treatment of recurrent Hodgkin's and non-Hodgkin's lymphoma.

Thousands of women and children residing near the Chernobyl reactor in the Soviet Union have been exposed to high levels of environmental radiation. Research into possible prophylaxis of radiogenic mammary carcinoma seems urgent in view of the continued likelihood of hazardous levels of mammary irradiation from a variety of sources in the future.

Two endocrine agents were selected for this study which have been widely used in clinical practice in recent years. Estriol (E3), the predominant estrogen secreted during pregnancy, has been administered in Europe to many women for relief of menopausal symptoms.[12,13] It has been well tolerated in doses as high as 6 to 20 mg (21–69 mm) daily orally in premenopause women.[14,15] Tamoxifen (TAM) was selected for comparison since it has been very

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effective in treatment of menopausal and postmenopausal breast cancer patients with receptor-positive tumors, with few serious side effects. It is representative of synthetic estrogen antagonists that might block the cocarcinogenic activity of endogenous estradiol 17β shown to promote radiogenic mammary carcinoma in some types of in-bred rats like the Sprague-Dawley.[9,10] Both of these estrogens inhibit promotion of chemically induced cancer in Sprague-Dawley females by quite different mechanisms.[16-18]

In this investigation we compare continuous monthly administration of E3 or TAM for possible inhibitory activity upon promotion of radiation-induced carcinogenesis. In addition, we evaluate only 6 months of prophylactic therapy using 17 alpha ethinyl estriol (EE3) which has greater estrogen agonist activity than E3.[19] Finally, a comparison was made with 6 months' treatment with 17 alpha ethinyl estradiol 17β (EE2) to ascertain whether the ethinylated native estrogen of the rat and women might have similar activity upon cancer promotion as E3.

Materials and Methods

The methods used were similar to those previously reported.[18,20,21] Virgin female Sprague-Dawley rats were obtained from Sasco, Inc. (Omaha, NE) when they were 40 to 50 days old. They were individually housed in suspended wire mesh cages at 22 to 23.5°C with a 12-hour light–dark cycle. Relative humidity was kept between 30% and 50%. The rats were fed Purina Rodent Chow (St. Louis, MO) no. 5002 with a 20.1% protein content and a 4.5% fat content. Distilled water was provided for their consumption. The rats received for each experiment were irradiated on the day after receipt by a cobalt 60 (^{60}Co) gamma source delivering 1.0 Gy/minute at 80 cm from the dorsal aspect of the rats. The rats were held in shallow compartmented plastic cages during irradiation with only two to four rats per compartment. After the first six experiments, a built-up bolus phantom was placed in the delivery path designed to maximize homogeneity of radiation absorption in the region of the ventrolateral mammary gland area to the desired 3.5 Gy. This minimized over-penetration of the target by the high-energy gamma radiation.

After exposure, the rats were randomized into control and treatment groups of eight to 26 each. Initially, hormonal therapy with E3 was begun 1 to 3 days before irradiation, and was later changed to 13 to 15 days after irradiation. Estriol (Sigma, Inc., St. Louis, MO) was compressed in 10% concentration into pellets composed of crystalline sodium chloride (NaCl) in a Forbes pellet press.[22] These pellets were 1 mm in diameter and 1 to 2 mm in length, weighing 6.38 mg ± 1.75 SD mean. Eli Lilly, Inc. (Greenfield, IN) supplied EE3 for investigation, and Sigma, Inc. supplied EE2.

Tamoxifen was prepared from Novaldex tablets manufactured by Stuart Pharmaceuticals (Wilmington, DE). These 10-mg tablets were cut into quarters for subcutaneous implantation. They contained carboxymethyl cellulose, calcium magnesium stearate, mannitol and starch in addition to tamoxifen citrate.

Pellets or tablet sections were implanted subcutaneously in the anterior dorsal area each month using light ether anesthesia. This procedure was shown to have no effect on the rates of induced mammary carcinogenesis previously.[20,21,23] Therefore sham implantation, or placebo controls were not employed. Nonirradiated female Sprague-Dawley rats observed over their natural life span rarely develop mammary carcinomas under the age of 500 days, so that nonirradiated controls were not included in any of our experiments.[10,24,25]

Rats were weighed and examined every 10 to 14 days during their natural life span after radiation by one of the authors. The date of the first palpable tumor which was usually 3 to 4 mm in diameter was recorded, and the site and size in two dimensions. Biopsies were performed on persistent and growing tumors within 2 to 4 weeks of discovery, at which time the rats were killed by ether anesthesia and underwent necropsy. In a single experiment (Exp. 9) the tumor was resected under anesthesia, the wound closed with stainless steel clips, and the rat allowed to survive as long as possible. Uteri were dissected free of adnexa and weighed in healthy rats with neoplasms. Tumor-free rats were observed until death from natural causes at which time they underwent necropsy; rats near death were anesthetized to obtain better-preserved necropsy specimens.

Ten percent formalin-fixed biopsy tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were interpreted on a double-blind basis by a single individual (H.M.L.) using published pathologic criteria of rodent mammary neoplasia.[26]

Rat mammary carcinomas rarely metastasize, and after irradiation stromal reaction to neoplasia is often greater than that observed after chemical induction. Our criteria for carcinoma were based upon the degree of anaplasia exhibited by the neoplastic epithelium, amount of disorder observed in glandular structure throughout the specimen, and the proliferative activity of the latter. Benign neoplasms, usually adenofibromas, exhibited uniform acini similar to those in nonirradiated rats, with 80% to 90% connective tissue components. Benign tumors may or may not be increased by radiation.[9-11]

The incidence rates for mammary carcinoma in control and treated groups were compared after all rats had been sacrificed for tumor or had died of nonneoplastic cause, using chi-square analysis with correction for small numbers. Tumor-free rats dying within 6 months after radiation exposure were censored from the data. Life-table

analysis was performed of the rate of carcinoma development in control and estrogen-treated groups to determine significance of differences; another test used was the Mann-Whitney U test for significance of increased rat-days free of mammary cancer experienced by the treated groups.

Results

In 142 controls, 93 rats developed mammary carcinomas after irradiation, in the time intervals shown in Figure 1. Censored rats were excluded from the analysis, as were rats developing nonmammary malignant neoplasms. Only 16% of tumors became palpable within 6 months after radiation exposure. Two thirds of mammary carcinomas appeared more than 300 days postirradiation, at which time there was a significant increase in the percentage of biopsy tissues with cancer ($P < 0.001$ by t test). Some carcinomas were discovered only at necropsy after death from other causes (usually pneumonia).

Six initial experiments in which E3 administration was begun 1 to 3 days before irradiation induced an insignificant reduction in mammary carcinoma incidence (Table 1). In a single group, delaying estriol therapy for 5 days after irradiation also failed to affect cancer incidence. When we observed in other ongoing studies that delaying estriol therapy for 2 to 4 weeks after cancer induction by each of two chemical carcinogens (procarbazine, 7,12 dimethylbenz(a)anthracene) resulted in maximal cancer prophylaxis, we revised our timing of hormonal therapy after irradiation accordingly.

In two of three additional experiments in which E3 or EE3 therapy was delayed 13 to 15 days after irradiation a significant reduction in mammary carcinogenesis was achieved (Table 2, Figs. 2 and 3). Life-table analysis revealed significant reduction in carcinogenesis after 12 months in these experiments, persisting as long as 16 months in the eighth trial (Fig. 2). There was a 42% reduction in final mammary carcinoma incidence in the pooled results of the two experiments using E3 ($P < 0.05$ by chi-square). Mean and median rat survival was longer in the treated groups because of 53 to 95 day delay in the mean time of mammary tumor detection (Table 2, Figs. 2 and 3). Frequency of mammary carcinomas including synchronous multiple tumors was reduced by 33% to 63% after E3 and EE3 therapy.

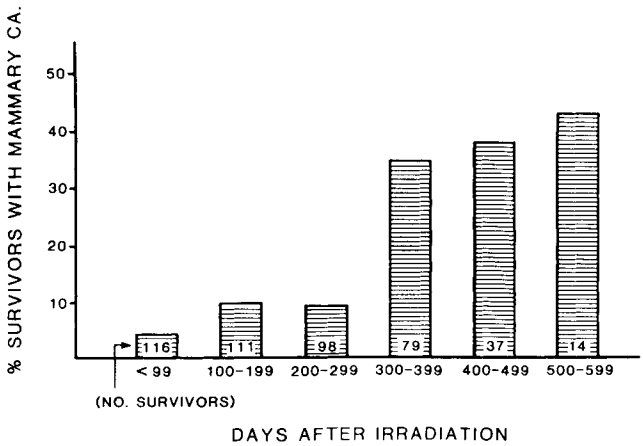

FIG. 1. Time of detection of palpable mammary carcinoma after irradiation of 142 control rats in Experiments 1 through 11. Excluded were seven censored rats, 18 with nonmammary epithelial malignant tumors, and one without pathologic confirmation. Two rats surviving over 600 days had mammary carcinoma. Only 7.8% of rats that underwent biopsy before 300 days had mammary carcinoma, and 38.3% of those surviving longer, $P < .001$ by t test.

TABLE 1. Incidence of Mammary Carcinomas After Estriol Treatment Begun Within 5 Days of Irradiation

	Controls			Treated				
Experiment	Mean time to tumor (d)	Incidence	Frequency per 1000 rat-days	Initial dose	Mean time to tumor (d)	Incidence	Frequency per 1000 rat-days	Significance of difference
1	406	7/12	1.72	3 d before Rx	409	10/21	1.38	NS
2	333	4/8	1.45	2 d before Rx	409	7/13	1.86	NS
3	112	4/6	5.0	2 d before Rx	275	1/4	0.86	NS*†
				2 d before Rx	272	4/8	1.99	NS†
4	377	6/10	2.16	2 d before Rx	439	4/10	1.29	NS
5	378	4/12	1.19	3 d before Rx	375	3/12	0.71	NS
				3 d before Rx	322	3/13	0.83	NS‡
6	408	4/6	2.45	1 d before Rx	316	7/12	2.29	NS‡§
				1 d before Rx	351	6/10	2.72	NS
				5 d after Rx	293	5/10	2.84	NS
Total	346	29/54 (53.7%)	1.90		337	50/113 (44.2%)	1.28	NS

NS: not significant; Rx: therapy.
* Estriol concentration 5% of pellets.
† Deaths from pneumonia limited survival; five rats censored from controls, six to eight censored from treated groups.
‡ Estriol-3-methyl ether implanted in 10.5% concentration in pellets.
§ Two rats censored from controls.

TABLE 2. Incidence of Mammary Carcinomas After Estriol Treatment Delayed for 13 to 15 Days

	Controls			Treated				
Experiment	Mean time to tumor (d)	Incidence	Frequency per 1000 rat-days	Initial dose	Mean time to tumor (d)	Incidence	Frequency per 1000 rat-days	Significance of difference
7	357	7/12	2.06	15 d after Rx	449	6/14‖	1.27	*
8	278	14/20	3.02	13 d after Rx	331	6/18‖	1.11	†§
9	258	23/26	3.07	15 d after Rx¶	353	14/22‖	2.06	‡§
Total		44/58 (75.9%)				26/54 (48.1%)		$P < 0.02$ by χ^2

Rx: therapy.

* After 12 mo cancer incidence in treated rats less by life table analysis, $P < 0.07$.

† From 12–16 mo cancer incidence less in treated rats by life table, $P < 0.02$.

‡ From 10–12 mo cancer incidence less in treated rats by life table, $P < 0.01$–0.04. Difference in rat-days free of cancer significant, $P = 0.06$ by U test.

§ Cancer incidence less than controls by χ^2, $P < 0.05$.

‖ Three rats censored from final results, dying tumor-free less than 180 d after irradiation.

¶ 17 alpha ethinyl estriol administered for only 6 mo.

In order to ascertain whether lifetime E3 prophylaxis was necessary, and whether its activity was specific or generic for estrogen agonist activity, only six monthly implantations of EE2 and EE3 were administered in a ninth experiment (Table 2, Fig. 3). Both estrogens delayed tumor onset similarly, with EE3-treated rats developing a 64% mammary cancer incidence, EE2-treated 75%, and controls 88% ($P < 0.05$ by chi-square only for EE3). Malignant nonmammary epithelial tumors occurred only once in the control and EE3-treated groups (4% incidence), but six rats (25%) developed other malignant neoplasms in those receiving EE2. Five of these rats were free of mammary cancer at necropsy. Six months' treatment with these estrogens reduced mammary cancer frequency by one third.

Estriol absorption from pellets and excretion rate in urine was measured isotopically previously, requiring about 2 weeks for completion.[23] Monthly E3 or EE3 implants did not significantly change uterine wet or dry weights at necropsy; uteri from moribund or dead animals were not weighed. Mean dry weight of control uteri was 135 mg, after E3 therapy 115 mg, with a SEM of 41 mg. Ethinyl estriol implants likewise did not alter uterine weights.[18,20,21,23]

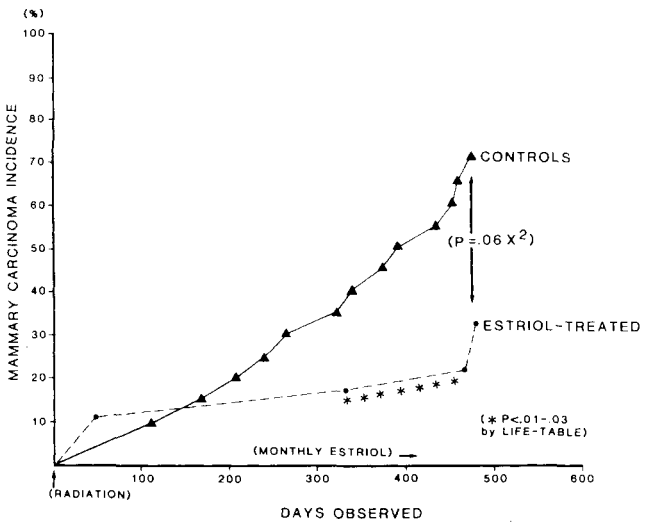

FIG. 2. Reduction in mammary carcinoma incidence achieved by continuous monthly estriol therapy begun 13 days after irradiation (Exp. 8).

The radiation dose of 3.5 Gy absorbed by rats in these experiments had no lasting effects upon pituitary–ovarian function, in accordance with earlier observations.[9–11] Uterine weights of irradiated control groups were compared to simultaneously observed control groups that had received 7,12 dimethylbenz(a)-anthracene (DMBA) as the carcinogen. The mean of mean uterine wet or dry weights of paired experimental control groups was 15% to 18% greater in the irradiated groups, in keeping with their generally older age when necropsy was done for tumor biopsy specimens, and somewhat greater average body weights.

Treatment with TAM was begun 14 to 15 days post-irradiation and was considerably more suppressive of mammary carcinogenesis than E3 (Table 3). Life-table analysis indicated a highly significant reduction in carcinoma incidence by the ninth to thirteenth month after exposure which persisted for the duration of the experiments. Final carcinoma incidence and the frequency of multiple synchronous carcinomas per 1000 rat-days were reduced by 80% to 83%. Therapy with TAM did not delay detection of neoplasia which appeared by 307 to 314 days, compared with 255 to 372 days in the controls. Duration of observation was similar in control and treated groups. A previous investigation reported the results of short-term TAM therapy on promotion of radiogenic carcinoma[16] which was not therefore further tested. The duration of absorption of TAM implants was not ascertained, except by the absence of any palpable residue at the time of the next implant in most cases. Necropsy uniformly revealed avascular small ovaries and thread-like uteri 1 mm in diameter. Uterine weights averaged 47% to 60% of the

control groups, significant ($P < 0.05$) in one experiment with the largest number of necropsy treated rats.

The incidence of benign mammary neoplasms was 6% to 14% in the control and treated groups throughout all 11 experiments in this study. Malignant nonmammary epithelial neoplasms occurred in 0% to 24% of control rat groups and in 3% to 12% of those receiving E3. Four months after irradiation, E3 treated rats averaged 10% less in body weight than controls, and those receiving TAM 13% less. Rats given ethinylated estrogens however weighed within 5% of the mean body weights of controls. No correlation has previously been observed between this degree of reduction in somatic growth and the antimammary carcinogenic activity of synthetic or natural estrogens or their metabolites.[20] Endocrine treatment induced no other adverse effects in these experiments.

Discussion

Based upon prior reports of long-term observation of Sprague-Dawley female rats from several sources, at least 80% to 90% of the carcinomas observed in this investigation appear to be radiogenic in origin.[11,24,25] Untreated Sprague-Dawley females housed in groups of two to ten per pan (permitting coprophagia) in the majority of cases develop benign or malignant mammary neoplasms toward the end of their natural lifespan.[25] Before 500 days observation, less than 10% developed mostly benign mammary tumors; about 50% of mammary carcinomas developed between 550 and 750 days postnatally, with a peak incidence at 700 to 750 days. Coprophagia with recycling of fecal estrogens[27] and bile acid derivatives was possible in the longest study.[25] Early killing of one of a pair of pan-housed rats significantly reduced mammary cancer incidence in the partner, suggesting an environmental influence.[25] Rats kept individually in wire mesh cages such as ours developed an 0.8% incidence of mammary adenocarcinoma after 20.8 months observation.[24]

The incidence of mammary carcinoma in our control groups rose from 54%, to 76% to 83% after addition of the bolus phantom to improve radiation dosimetry. Nevertheless, no changes were seen in the 6% to 14% rate of benign tumor development, during the time required for completion of the experiments, which was within the expected range for untreated rats of similar age.[9-11,24,25]

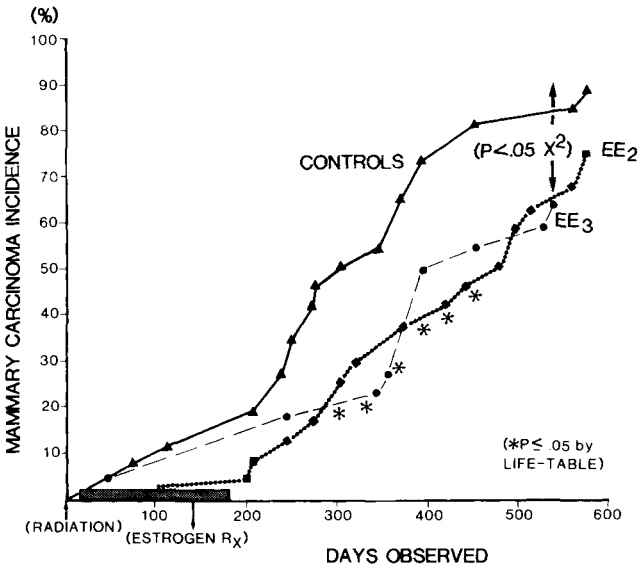

FIG. 3. Reduction in rate of mammary carcinoma development after 6 months of treatment with 17 alpha ethinyl estriol (EE3) or 17 alpha ethinyl estradiol (EE2), begun 15 days after radiation exposure.

No direct comparison was made in the same experiment of simultaneous and delayed estriol therapy after irradiation. The improved prophylaxis of cancer noted in sequential experiments between antecedent treatment and that which began 2 weeks after exposure was similar to the results found with delaying EE3 prophylaxis in DMBA-induced mammary carcinogenesis.[18,21] Both E3 and EE3 in 638-μg/month doses induce rapid prelactational lobuloalveolar differentiation of immature virginal glands. Estrogen-induced differentiation probably reduced the numbers of terminal ductules and end buds from which most chemically induced carcinomas are believed to arise.[28] Whether a similar mechanism may contribute

TABLE 3. Incidence of Mammary Carcinomas After Tamoxifen Treatment Delayed for 14 to 15 Days

	Controls			Treated				
Experiment	Mean time to tumor (d)	Incidence	Frequency per 1000 rat-days	Initial dose	Mean time to tumor (d)	Incidence	Frequency per 1000 rat-days	Significance of difference
10	255	12/15	2.97	14 d after Rx	314	2/13§	0.40	*‡
11	372	13/15	2.92	15 d after Rx	307	3/23‖	0.29	†‡
Total		25/30 (83.3%)				5/36 (13.9%)		

Rx: therapy.

* After 9 mo cancer incidence less in treated rats by life table analysis, $P < 0.03$.

† After 13 mo cancer incidence less in treated rats by life table, $P < 0.03$.

‡ Cancer incidence less than controls by χ^2, $P < 0.001$.

§ Two rats censored from final results, dying tumor-free less than 180 d after irradiation.

‖ One rat censored from final result for the same reason as above.

to the suppression of carcinogenic promotion of radiation-induced rat mammary carcinoma will require further investigation.

Six months of EE3 therapy was far less inhibitory of radiogenic mammary carcinoma than chemically induced neoplasms, where limited treatment was as effective as ovariectomy in prevention of DMBA-induced carcinomas.[18] In addition, EE2 induced the same lag in palpable tumor formation as EE3, and the final mammary carcinoma incidence was not significantly different between the two estrogenic treatments. Identical doses of EE2 were wholly ineffective in modifying either the rate of neoplasia development or the final incidence of chemically induced carcinomas.[18] Since only EE2 and not E3 or EE3 administration raise serum prolactin concentrations,[15,29] this lesser response of radiogenic tumor promotion to estriol suggests that circulating prolactin levels have little influence upon the damage that remains after irradiation in DNA templates, and the ensuing changes in receptor functions or growth factors involved in replication.

Cell proliferation such as that induced by estrogens in nulliparous mammary glands interferes with repair of extensive DNA injury resulting from 3.5 Gy absorption.[30-32] Both processes compete for the same limited pool of high-energy intracellular phosphate, and replication of unrepaired DNA templates can immortalize serious mutational changes promoting carcinogenesis. There is no data that we know of as to the duration of mammary DNA repair after irradiation; a minimum period of 10 to 14 days appears to be necessary for hemopoietic tissues to recover from acute radiation injury. A single experiment suggested that a 5-day treatment delay was insufficient, and further investigation would be of interest.

Mammary differentiation induced by pregnancy or endocrine therapy clearly inhibits promotion of chemically induced mammary neoplasma.[18,28] Differentiation inhibits promotion of a few other tumors possibly by blocking replication of transformed cells.[33] Ethinyl estriol was equally active as EE2 in rapidly differentiating virgin rat mammae in doses of 2 to 6 μg/day (6–18 nm/day) in a 28-day period.[18] Six months of EE2 and EE3 therapy similarly inhibited the rate of mammary tumor appearance and final incidence, supporting a generic estrogenic role in differentiating virginal gland structure (Table 2, Fig. 3). A six-fold increment in nonmammary malignant tumors occurred, however, in those receiving EE2, which is consistent with the cocarcinogenic role of E2 after irradiation.

The importance of this contribution of E2 to mammary carcinogenesis was shown in the value of TAM prophylaxis extended for the natural lifespan of radiated rats, which induced the greatest 83% reduction in cancer incidence (Table 3). When TAM was administered for 2 months after radiation in 50 to 200 μg/day (89–178 μm/day) doses, mammary tumors were decreased by 40% to 50% from control incidence after 259 days' observation.[17] Ovariectomy has also more markedly inhibited promotion of radiogenic mammary cancer.[11,34]

The biologic activities of TAM and E3 depend upon the integrity of estrogen receptor proteins in mammary glands and elsewhere, which are resistant to injury even by high radiation doses *in vitro* as well as *in vivo*.[35,36] Receptor functions were intact after 3.5 Gy whole-body radiation as shown by the decreased uterine weights and nearly complete tumor inhibition by TAM. Since E3 and EE3 inhibit pituitary luteinizing hormone (LH) secretion as well as EE2,[29] monthly administration of 638 μg of these estrogens intermittently suppressed estrus cycles and ovarian secretion of E2.[18] This led to an absence of uterine hypertrophy even after a year or more of endocrine therapy.

Ionizing radiation can induce mammary carcinoma with equal facility at different ages in female Sprague-Dawley rats.[10] However, in Japanese women survivors of nuclear bombs, the greatest increase in mammary cancer risk has occurred in those irradiated at the earliest age.[1] A steady decline in absolute and relative breast cancer risk occurred with increasing age at exposure. In survivors receiving 0.5 Gy or greater dose, women irradiated at age 10 to 14 years currently have more than twice the cancer risk as those older than 24 years, in whom a larger percent would have been parous. When only the chest received gamma radiation, the largest increase in breast cancer risk was in those aged 15 to 19 years when exposed; no excess risk was reported in those older than 30 years.[4] Radiation-induced premature amenorrhea was not likely in these women as a cause of decreased risk in older women, such as some of the Japanese women may have experienced.

Another difference between rodents and women in radiation induction of cancer is the temporal acceleration of mammary tumorigenesis in virgin female rats. Non-irradiated Sprague-Dawley females rarely develop carcinomas before the age of 1 year (1.2% incidence[10]).[24,25] At this age, our radiated females were showing a rapid increase in mammary carcinomas, with completion of many experiments within the next 3 months (Fig. 1, Tables 1–3). Japanese survivors of radiation from nuclear weapons have shown increased risk of breast cancer that follows the same age distribution pattern as nonradiogenic cancer; at any given age the increased risk was proportional to the underlying breast cancer proneness. At least a decade elapsed before excess cancer cases followed irradiation, or the women had to reach age 30 years for this to occur.

The 3.5-Gy radiation dose delivered to the nulliparous mammae of our rats was in the higher range of the estimated mammary exposure of exposed Japanese women, which 35 years later increased breast cancer risk up to ten-fold above that of unexposed women.[1] In postpartum

women who received 200 to 450 rads of x-radiation in the US, a three-fold increase in breast cancer occurred in the radiated breast compared to the contralateral breast or other controls, after a 29-year mean follow-up.[3] This increase in cancer risk in a white population appears minimal considering the five-fold higher spontaneous breast cancer incidence in the US, raising the question of some risk reduction by the lactational differentiation of the radiated tissues. Our endocrine prophylaxis has been tested in rats who received appropriately high radiation exposure commensurate with historic experience.

Other investigators have concluded that the physiologic state of the rat had little influence upon radiation induction of mammary cancer, based upon the results of exposing virgin, pregnant, lactating, and postlactational females to 200 rad whole-body 250-kVp x-rays. Maximum follow-up was 314 days postirradiation, when no significant differences were observed either in incidence of fibroadenomas or of mammary adenocarcinomas.[37] However, in Wistar rats followed for 2 years after radiation significantly less mammary tumorigenesis was observed in those irradiated when pregnant, equal to that seen in unirradiated controls.[38] Rats secrete E2 as their principal estrogen throughout pregnancy,[39] which unlike E3 transforms mouse fibroblast 3T3 cells and is six-fold more carcinogenic in the hamster kidney.[40,41] Estriol impairs E2 receptor binding and hastens its nuclear release[42]; it is not bound to plasma sex hormone-binding globulin that sequesters 98% of E2 from tissue access in women.[43] During the last trimester of pregnancy E3 production exceeds E2 by ten-fold, leading to probable saturation of many maternal and fetal estrogen receptors by E3.[44] Estriol is metabolically inert aside from conjugation with sulfates or glucuronides, and unlike E2, is only minimally further oxidized *in vivo* to 2-OH E3. Several investigators have related estrogen 2/4 hydroxylase activity which yields 2-OH estradiol as the principal metabolite of E2 to transforming and carcinogenic activity; fluorination of estradiol at C2 blocked 2-hydroxylation and eliminated hamster renal carcinogenicity.[40,45,46] These differences between E2 and E3 are most important in the influential role that human pregnancy and its timing play in affecting risks of mammary cancer.[47] Postpregnancy increase in E3 production, and 50% reduction of plasma prolactin may also contribute to risk reduction after pregnancy.[48,49]

Parenteral E3 or EE3 therapy can simulate the altered E2/E3 tissue ratios seen in human pregnancy, thereby safely differentiating immature mammary duct epithelium in nulliparous females. This can be done without interfering with rat estrus or inducing uterine hypertrophy, in lower milligram/kilogram daily doses than are well tolerated orally by healthy premenopausal women.[14,15] This method of cancer prophylaxis provides an alternative to functional castration as a means of reducing the cocarcinogenic activity of E2 in radiogenic as well as in nonradiogenic rat mammary cancer. Whether it can be utilized in other species requires further investigation.

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