Radiation-induced Mammary Carcinogenesis in Virgin, Pregnant, Lactating, and Postlactating Rats

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ABSTRACT

Little attention has been paid to the influence of the reproductive condition of the rat at the time of irradiation upon X-ray-induced mammary carcinogenesis. At 12 weeks of age, 160 virgin female Sprague-Dawley rats were mated, and another 140 were maintained as virgins. On Days 14 to 16 of pregnancy, 36 mated females and 40 virgins were whole-body irradiated with 200 R of X-rays. On Days 2 to 5 postpartum, 40 additional mated females and 40 additional virgins were irradiated. At this time, all pups were removed from their mothers; 2 weeks later, 40 additional parous females and 40 virgins were irradiated. The remaining animals: parous females; virgins; and mated, but nonparous females were kept as unirradiated controls. Breast tissue biopsies were taken from 25% of the animals in each irradiated group on the day of irradiation, and the biopsies were examined microscopically for mammary tissue development. The experiment was terminated 10 months after irradiation. Despite marked differences in mammary tissue development among the groups at the time of irradiation, there were no significant differences among the irradiated groups for the incidence of rats with mammary adenocarcinomas or the number of mammary adenocarcinomas per rat. There were no significant differences among the irradiated groups for the incidence of rats with mammary fibroadenomas. There was an increase in the number of fibroadenomas per rat which was associated with increased age at the time of death. In contrast to mammary carcinogenesis produced by polycyclic aromatic hydrocarbons in previous studies, in the current investigation the physiological status of the host at the time of irradiation had little effect on: (a) the final incidence of mammary neoplasia; (b) the number of mammary adenocarcinomas produced; or (c) the type of mammary neoplasms produced.

INTRODUCTION

Mammary carcinogenesis induced by polycyclic aromatic hydrocarbons in female Sprague-Dawley rats has been shown to be dependent on the age and physiological condition of the animal at the time of p.o. or systemic carcinogen administration (1, 2, 9, 10, 17, 18, 22). Except for very young females, radiation-induced mammary carcinogenesis has not been shown to be age dependent in virgin females (8, 17). However, the effect of physiological status of the rat at the time of irradiation has received relatively little attention (18). Therefore, we undertook a study to compare the effects of irradiation during pregnancy, lactation, postlactation, and virginity upon mammary carcinogenesis in Sprague-Dawley rats.

MATERIALS AND METHODS

At approximately 12 weeks of age, 160 virgin female Sprague-Dawley rats (Taconic Farms, Germantown, N. Y.) were placed into 80 plastic tubes. Each tube contained 1 male and 2 females. Another 140 virgin females of the same age and source were kept isolated from the matings in another animal room. All rats were maintained on commercial rat chow and water ad libitum, under 12 hr (8 a.m. to 8 p.m.) fluorescent light per day, at 22.5 ± 1° (S.D.) and 55 ± 5% humidity.

Mating success was checked by gross examinations for vaginal plugs and microscopic examinations for sperm in vaginal smears at 9 a.m. and at 4 p.m. daily. Upon evidence of impregnation, pairs of the pregnant females were placed in cages in another animal room. At Days 14, 15, and 16 postfertilization, a total of 36 pregnant females (Group 1) were whole-body irradiated with 200 R of 250-kVp X-rays (21), along with a total of 40 virgin females (Group 2). Two to 5 days after parturition, another 40 lactating females (Group 3) and 40 virgins (Group 4) were irradiated. Gross external observation of milk in the stomach of the pups was used to detect lactation and milk letdown. At this time, the pups were removed from all the females that gave birth. Fourteen days later, another 40 of the parous rats (Group 5) and 40 virgins (Group 6) were irradiated. Another 28 parous rats (Group 7), 20 virgins (Group 8), and 12 mated (Group 9) but nonpregnant rats were maintained as unirradiated controls.

For each irradiated rat, time was reckoned from the day of irradiation, Day 0; for the unirradiated controls, the first day of irradiation of the pregnant females was used as Day 0. One hr after irradiation, 10 females from each of the irradiated parous and irradiated virgin control groups were subjected to light ether anesthesia. A biopsy, approximately 10 sq mm, was taken from the breast tissue medial to the nipple of the right lower quadrant of each anesthetized rat. The biopsies were fixed in formalin, defatted, stained, dehydrated, cleared, and mounted unsectioned for evaluation of mammary tissue development (24). The samples were scored from 1 to 5 at 0.5-unit intervals, with the larger values representing greater development (27). Because of the variation, both mean values and range are given in Table 1.

The rats were kept for 293 to 314 days postirradiation (Table 1). Although the mean time in the experiment was almost the same for all irradiated groups, Groups 1 and 2 were 2 to 4 weeks younger than were Groups 3 through 6 at the time of irradiation. Therefore, Groups 1 and 2 were 2 to 4 weeks younger than the other irradiated groups when the rats were killed. Each rat was identified by a numbered ear tag. Starting at 4 weeks postirradiation, the animals were palpated on a weekly basis for tumors. The anatomical location of each mammary tumor was recorded using the nipples as reference points. When approximately 2 cm in diameter, individual mammary tumors were removed from the rats under light ether anesthesia. Upon removal of a tumor, each rat was returned to the experiment. Any tumor recurring within 12 weeks at the site of removal of a previous tumor of the same type was not counted. Hematoxylin- and eosin-stained sections of the mammary tumors were classified as MACs3 or FAs according to criteria

1 Research carried out under the auspices of the United States Department of Energy under Contract DE-AC02-76CH00016. Portions of this research were presented at the 72nd Annual Meeting of the American Association for Cancer Research, Washington, D. C., April 27 to 30, 1981 (7).

2 To whom requests for reprints should be addressed.

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3 The abbreviations used are: MAC, mammary adenocarcinoma; FA, mammary fibroadenoma; i.g., intragastric; DMBA, 7,12-dimethylbenz(a)anthracene.
Mammary gland neoplasia produced by whole-body X-irradiation of pregnant, lactating, postlactating, and virgin Sprague-Dawley rats

<table>
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<th>Group</th>
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<td>3. Lactating (2–5 days)</td>
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^a Graded to nearest 0.5 on biopsies taken on the day of irradiation.
^b 200 R of 250-kVp X-rays, whole body.
^c Group 3 greater than Group 1 (p < 0.05, t test).
^d Group 2 versus Group 4 (0.05 < p < 0.1, t test).
^e Groups 2 and 5 less than Group 1 (p < 0.05, t test).
^f Tumors per rat for Groups 3 and 5 greater than for Group 1 (p < 0.05, t test).

RESULTS

At the time of irradiation, there were clear-cut differences in the development of mammary glands among groups, and there was considerable variability within the groups that were not pregnant or lactating. In the rats of Group 1, ductal and lobuloalveolar development approached the maximum previously described for mammary glands of mid-to-late-pregnant rats (27). The mammary glands of postlactating rats (Group 5) presumably were variably regressed to give an average score that was slightly higher than the scores from the variable breast morphology of the virgins (Groups 2, 4, and 6). The number of offspring, their body weights, and suckling did not appear to be significantly affected in the irradiated pregnant rats as compared to the other parous rats.4

As expected from previous studies (20), in this investigation, MACs tended to appear earlier than did FAs. The incidence of rats bearing MACs and the number of MACs per group or per rat were not significantly different among the irradiated groups. However, the time to detection of MACs was significantly shorter for pregnant rats (Group 1) than for lactating rats (Group 3).

Although the incidence of rats bearing FAs appears to be small for the pregnant rats (Group 1), there were no significant differences in incidence for any of the irradiated groups. The number of FAs per group and FAs per rat tended to increase from the groups irradiated earlier (Groups 1 and 2) to those irradiated later (Groups 3, 4, 5, and 6), with significantly fewer FAs per rat in the pregnant rats than in either the lactating or postlactating rats. Among the irradiated experimental groups, there was a trend towards decreasing latency of FA detection from the groups irradiated earliest (Groups 1 and 2) to those irradiated later (Groups 3 through 6). Among the irradiated groups, the only significant differences for days to detection of first FAs were between the pregnant rats (Group 1) and their controls (Group 2) and between the pregnant rats and the postlactating rats (Group 5).

DISCUSSION

At the time of irradiation, there were clear-cut differences in mammary gland development between groups and considerable variability within some groups. The current investigation found no relationship for the status of mammary glandular tissue in female Sprague-Dawley rats (and, by extension, the physiological status of the rats) at the time of X-irradiation either to: (a) the resulting incidence of mammary neoplasia; (b) the number of mammary adenocarcinomas produced; or (c) the type of mammary neoplasm produced. Neither pregnant, nor lactating, nor postlactating irradiated rats were different from their respective irradiated virgin controls. Furthermore, analyses of subdivisions of the irradiated mated rat groups (e.g., Group 1 irradiated on Day 14, 15, or 16) showed no differences from each other or from the totals for each group. The current study confirms and extends the results of a previous investigation by Shellabarger (18) in which inbred Sprague-Dawley rats, irradiated during lactation, did not respond differently from their irradiated-virgin controls.

In other studies, no relationships were found between the age of virgin Sprague-Dawley females at the time of irradiation and mammary carcinogenesis, except for immature rats (8, 17).

The results described above contrast markedly with those obtained with p.o. polycyclic aromatic hydrocarbons, where a
critical-age treatment period has been demonstrated for maxi-
mal mammary carcinogenesis in virgin female Sprague-Dawley
rats (1, 2, 9, 10). Furthermore, mammary neoplasia was re-
duced when pregnant or lactating rats received p.o. polycyclic
aromatic hydrocarbon treatment (2, 3, 10, 18). Moon (13)
found that a single pregnancy, without lactation, prior to i.g.
administration of DMBA produced maximum protection against
mammary carcinogenesis. Dao (4) reported that prior preg-
nancy inhibited mammary tumorigenesis by both p.o. DMBA or
dDMBA applied directly to the breast tissue. The relationship of
mammary gland morphology and development in Sprague-
Dawley females to chemically induced carcinogenesis has
been elegantly described by Russo and Russo (16), and similar
observations have been made on other rat strains (6). These
investigators have concluded that maximum MAC induction
occurs by p.o. DMBA administration at a stage of development
that is predominated by the presence of terminal end buds,
which are in turn associated with extensive cellular prolifera-
tion. However, in contrast to the observations on parous rats
(4), recent publications (14, 22) reported that direct application
of DMBA to mammary glands in Sprague-Dawley females did
not produce a lesser incidence of MACs when the treatment
was given to older rats. The youngest rats used in those studies
were of the age in which p.o. or systemic DMBA administration
would be expected to produce a maximal response. Those
observations (14, 22) support the view that metabolism and
clearance rates are important factors in mammary carcino-
genesis induced by p.o. polycyclic aromatic hydrocarbon treat-
mant (2, 5, 12, 18). It is likely that the critical period for maximal
mammary carcinogenesis induction by p.o. polycyclic hydro-
carbons depends both on the interactions between the devel-
opment condition of the mammary tissue itself and on the
metabolism and clearance rates of the polycyclic hydrocar-
bons. It remains to be determined as to which of these 2 factors
is predominant in polycyclic hydrocarbon-induced mammary
carcinogenesis.

We have insufficient information to discuss differences in the
inductive action of chemicals and radiation in rat mammary
carcinogenesis. In the case of radiation, it is possible that
induction is occurring in a specific stem cell population which
is maintained throughout the life and reproductive changes in
the rat, while chemical carcinogenesis is more related to the
number, rate of turnover, and types of differentiated mammary
gland cells.

Specific links between chemical carcinogens and human
breast cancer have not been established, although we do not
exclude the existence of such relationships. Nevertheless,
there is considerable evidence for radiation induction of breast
cancer in humans, and the similarities to radiation-induced
mammary carcinogenesis in rats have been described else-
where (19). However, it is of some interest to note that in
irradiated women the risk per rad was not very different be-
tween women who were lactating at the time of radiation and
women who were not lactating at the time of radiation (11).

The dose of whole-body X-irradiation administered to the
rats in the current experiment was selected to provide minimal
interference with reproductive function, while producing a sig-
nificant mammary carcinogenic response in a relatively short
time (18, 20). The experiment was successful in both aims, as
evidenced by the comparisons of birth, lactation, and tumor
results between the irradiated parous rats (Groups 1, 3, and 5)
and the unirradiated parous rats (Group 7).

We have no explanation for the apparently earlier MAC
responses in the irradiated pregnant rats and their irradiated
virgin controls (Groups 1 and 2), as compared to the irradiated
lactating rats and their irradiated virgin controls (Groups 3 and
4). These relationships did not hold up under trend analyses.

In the current investigation with Sprague-Dawley females,
which are responsive to X-irradiation in terms of mammary
neoplasia, the apparently increased FA responses in Groups
3, 4, 5, and 6 in comparison to Groups 1 and 2 are readily
explicable. As confirmed in the present investigation, FAs tend
to appear later than MACs (20), and the increased FA re-
sponses may be related to the absolute age of the rats at the
time of death, rather than to time after irradiation. This is due
to the experimental design where time to detection of FAs was
counted from the day of irradiation, so that groups which were
irradiated later were also older when killed. Therefore, one
might expect to find more FAs in those groups irradiated later.
It is possible that keeping Group 1 until the same “absolute
age” as groups killed later would have resulted in a similar final
cumulative incidence of rats with FAs between Group 1 and
the other irradiated groups. The relationship of absolute age at
death to FA responses may also be an explanation for the
apparently shorter latency of FA responses in those groups
that were irradiated later. One way to test these suggestions is
to change the order in which the experimental groups are
irradiated. Nevertheless, for all the irradiated groups, the ex-
periment confirms previous observations that radiation in-
creases FA responses in Sprague-Dawley females as com-
pared to unirradiated controls (19).

In a previous investigation on Wistar rats studied for 2 years
after 270 R of X-irradiation at 121 days of age, Reincke et al.
(15) detected a significantly lower incidence of “benign,” i.e.,
FA, mammary tumor-bearing females for rats irradiated while
pregnant, as compared to the incidence in irradiated virgins.
The incidence for benign mammary tumor-bearing Wistar fe-
males irradiated when pregnant was not different from the
incidence in unirradiated controls. The study differed from the
current investigation in several details. The study of Reincke et
al. (15) was over a 2-year follow-up, while our study was
completed in 10 months after irradiation. Tumors were removed
as they appeared in our study but not in the study of Reincke
et al. (15), and we used Sprague-Dawley rats while they used
Wistar rats. However, the data suggest that Wistar rats are less
sensitive to X-radiation than are Sprague-Dawley rats since the
maximum incidence of Wistar females with “malignant,” i.e.,
MAC, tumors was only 4 of 45 irradiated virgins.

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