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Cancer Letters 148 (2000) 121–126

CANCER Letters

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Effect of constant light on DMBA mammary tumorigenesis in rats

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Received 17 May 1999; received in revised form 16 August 1999; accepted 18 August 1999

Abstract

A study of light, and mammary tumorigenesis was conducted in rats. One-hundred female Sprague–Dawley rats were divided by weight into two groups. One group was exposed to constant light (LL) from 26 days of age, and the second group was exposed to 8 h light and 16 h dark per day (LD). Both groups received an 8 mg dose of a chemical carcinogen, dimethylbenzanthracene (DMBA) at 52 days of age. At 13 weeks post-DMBA, there were significantly fewer mammary tumors in the LL group compared with the LD group. Constant light was clearly demonstrated to have a profound effect on mammary tissue development. Although virgin, the majority of the LL rats (29/50) had gross evidence of lactation at 141 days of age. None of the LD rats (0/50) showed evidence of milk production. These results suggest that constant light not only substantially accelerated mammary gland development, but pushed development of the tissue past the stage normally observed in virgin animals (to the lactation stage).

Keywords: Mammary gland development; Light exposure; Mammary cancer; Dimethylbenzanthracene; Carcinogenesis; Rats

1. Introduction

Breast cancer incidence rates are high and rising in the USA and appear to be much more common in industrialized than in non-industrialized countries [1]. As common as breast cancer is in the industrialized World, risk continues to increase [2]. The historically low rates of breast cancer in Japan have been increasing rapidly in recent decades [3]. The reasons for the increases in breast cancer risk are not well understood [4]. One possible contributor may be 'light pollution' during the night, and artificial light during the day [5,6]. In the evolutionary past the environment consisted of dark nights and bright, full spectrum days, whereas the modem environment is comprised of lighted nights in homes, and dim, spectrum-restricted 'days' inside buildings where most people now work. Indeed, the 'built environment' is the predominant environment in the industrialized world. This change in lighting may change our circadian physiology, in particular normal cycling of melatonin and other hormones, leading to early menarche and elevated circulating estrogen and prolactin [7]. Early menarche and elevated sex hormones are known to increase breast cancer risk [8,9].

There is very limited direct evidence on the relationship between light and breast cancer, and little ongoing work. In 1964, Jöchle reported that the development of spontaneous mammary tumors in C3H-A mice was increased by constant illumination [10]. Early experiments wherein rats were initiated with

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0304-3835/00/$ - see front matter
PII: S0304-3835(99)00320-1

high doses (20–30 mg) of dimethylbenzanthracene (DMBA) and exposed to extended or constant-light photoperiods yielded mixed results [11–13]. Later, Shah et al. [14] and Mhatre et al. [15] exposed the animals to continuous light from before birth, through the length of the study. Using a dose of 10 mg DMBA per 100 g body wt. of the rats, they reported that constant light increased DMBA-induced mammary tumorigenesis. At 55 days of age, rats exposed to LL showed a greater concentration of terminal end buds and alveolar buds in mammary tissue than was observed in rats exposed to a 10-h light:14-h dark regimen. Constant light animals also showed greater DNA synthesis activity in the mammary tissue, and higher levels of circulating prolactin. In rats, precocious puberty resulting from light-at-night (LAN) may serve to increase or decrease sensitivity to an acute exposure to carcinogens depending on the timing of that exposure. We embarked on this research effort to examine the effects of various light exposure regimens on mammary tissue development and tumorigenesis.

2. Materials and methods

Female Sprague–Dawley rats, 24 days of age, were obtained from Charles River Laboratory (Raleigh, NC). Before initiation of the study, 5 rats were randomly selected for parasite evaluation and gross observation for evidence of disease. Serological evaluation for Sendai virus, pneumonia virus of mice, rat coronavirus/sialodacro-adenitis virus, Kilham rat virus/H-1 virus failed to reveal any abnormalities. Animals were housed five per polycarbonate cage ($23'' \times 15'' \times 8''$) on hardwood bedding (P.J. Murphy Forest Products, Montville, NJ). Cages were changed twice weekly and water and NIH-07 open formula pelleted diet (Ziegler Brothers, Inc., Gardners, PA) were available ad libitum. Temperature was maintained between 22 ± 2°C, and relative humidity between 50 ± 15%. The light level from 40 W Trimline fluorescent bulbs averaged 178 and 175 lux (SDs: 7.4 and 7.8, respectively) between the two rooms. The irradiance of these bulbs was measured using a spectroradiometer (PhotoResearch, model 650, Chatsworth, CA). Light levels varied between 250 and 120 lux at the front and rear of individual rat cages.

The study animals were randomized, based on weight at 26 days of age and separated into two groups of 50 animals. One group was housed with a light-dark cycle of 8:16 (LD; lights on from 8 am to 4 pm) and the other group maintained in a constant light environment of 24:0 (LL). The 8L:16D photoperiod is characterized by an extended nocturnal period and a lengthened duration of melatonin production [16,17]. Such a scheme was chosen to enhance the light exposure differences between animals in constant light versus those in the cycling (control) photoperiod. At the time of group assignment, the average weight was equivalent in the two groups at 58 g/animal (SD = 7.9). Cages were rotated on each rack once per week to minimize inhomogeneity in light level.

In previous experiments in our laboratory, it was determined that 8 mg of DMBA yields tumors in about 40% of Sprague–Dawley rats by 141 days of age. Therefore, in this study all rats were dosed with 8 mg DMBA (TCI America, Portland, OR) in sesame oil intragastrically at 52 days of age (between 9 and 10 am). Subsequently, the rats were palpated weekly and masses were located and recorded by specific mammary gland location (L1–L6 and R1–R6). Tumor size was determined by comparing the masses with wooden spheres of defined size (0.5–5 cm). Two individuals each palpated half of the rats each week, alternating groups of rats. When there was a discrepancy between the previous week in number or size of the masses, then both individuals palpated the animal and resolved the discrepancy. Presence and location of tumors were confirmed at necropsy.

At 141 days of age, rats were euthanized with $\sim$70% CO_2 and necropsied. The skin with the breast epithelium was reflected from the rat by dissecting the fascia above the musculature. Any mammary tumors were measured (largest and smallest diameter) and the size recorded.

Body weights were evaluated by repeated measures analysis of variance. Tumor palpation results repeated over time (tumor incidence and total tumors) were analyzed by a non-parametric Friedman repeated measures analysis of variance on ranks. Dunnett's test was used to delineate intergroup differences. Tumor necropsy results were analyzed by the non-parametric Mann–Whitney rank sum test [18].

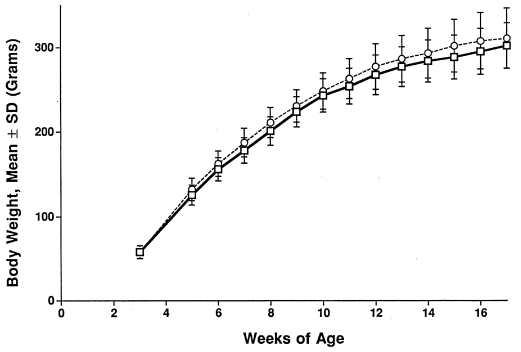

Fig. 1. Average body weight (mean ± SE) of rats exposed to constant light beginning at 26 days of age, LL group (- - - ○- - -); or to normal lighting, 8:16, LD group (□). Both groups were gavaged with DMBA (8 mg) at 52 days of age. The growth curves of the two groups were not statistically different ($P > 0.05$).

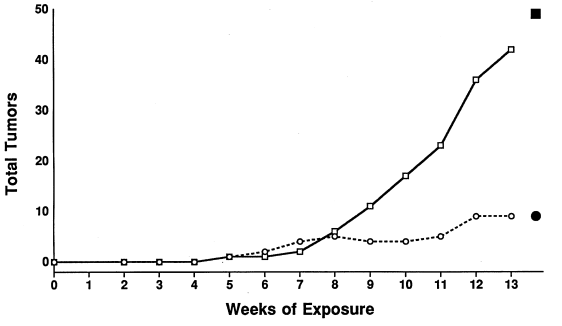

Fig. 3. Total palpable masses in rats exposed to constant light beginning at 26 days of age, LL group (- - - ○- - -); or to normal lighting, 8:16, LD group (□). Both groups were gavaged with DMBA (8 mg) at 52 days of age. The total number of confirmed tumors at necropsy in the LL group (○) and LD group (□). The groups were significantly different ($P < 0.01$) by analysis of variance.

3. Results

No significant differences were observed in the growth or growth rates observed in the exposed and control animals (Fig. 1). At time of DMBA administration (52 days of age), the LL group average weight was slightly higher than the LD group (187 vs. 178 g; SD = 17 g).

Weekly palpation of rats identified the first tumors at 5 weeks post DMBA administration in the LD animals. Palpable masses were first observed in the LL animals at 7 weeks post DMBA and from that point there was an increase in proportion of tumor bearing rats in both groups. There was a clear difference between the groups in the number of animals developing tumors as the study progressed, with a much higher proportion of the LD animals having tumors (Fig. 2). In addition, the total number of tumors was markedly increased in LD animals (Fig. 3). Analysis of variance showed a significant difference in the palpation curves between LD and LL groups for tumor incidence (% rats with tumors) ($P < 0.05$) and for total tumors ($P < 0.01$). The number of rats with palpable mammary tumors in the LL group was significantly less than that of the LD group at weeks 12 and 13 ($P < 0.05$). Total mammary tumors of the LL group were also significantly less compared with LD animals at weeks 11 ($P < 0.05$), 12 and 13 ($P < 0.01$).

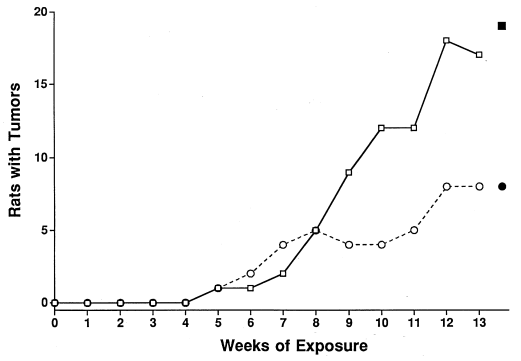

Fig. 2. Number of rats with palpable masses exposed to constant light beginning at 26 days of age, LL group (- - - ○- - -); or to normal lighting, 8:16, LD group (□). Both groups were gavaged with DMBA (8 mg) at 52 days of age. The number of rats confirmed with tumors at necropsy in the LL group (○) and LD group (□). The groups were significantly different ($P < 0.05$) by analysis of variance.

Upon terminal sacrifice, at 141 days of age (13 weeks post-DMBA), gross inspection of mammary tissue in each rat revealed tumors in eight rats in the LL group and tumors in 19 rats in the LD group (nine and 49 tumors, respectively). The number of tumors per tumor-bearing animal was 1.1 in the LL group and 2.6 in the LD group. These differences in number of animals with tumors ($P < 0.05$), in tumors per animal ($P < 0.05$), and in total tumor yield ($P < 0.01$) are all statistically significant. In addition, there was clear evidence of lactation nodules (milk sacs) in 29 of the LL rats and none of the LD rats (Table 1).

Examination of the anatomical location of tumors

Table 1
Number of rats (of 50) with tumors, abdominal/inguinal tumors, or lactation nodules at necropsy

Exposure group	No. of rats with tumors	No. of rats with abdominal tumors	No. rats with lactation nodules
LD	19	12	0
LL	8	1	29

revealed that 63% of LD rats with tumors had tumors located in the abdominal or inguinal regions (Table 1), and only 13% of LL rats with tumors had tumors located in comparable sites, suggesting that the abdominal and inguinal regions were less susceptible to tumor formation in the LL group. In addition, four rats in the LL group had a lobular cyst while none of LD rats had evidence of lobular cysts.

4. Discussion

Rat mammary glands are paired along the ventral aspect of the animal, with one pair in the cervical region, two in the thoracic region, one in the abdominal region and two in the inguinal region. Normally (in 12:12 light-dark cycle and no carcinogen administration), female rats first begin estrus at 32–35 days of age. As the mammary gland develops, terminal end buds (TEB) begin to appear and subsequently evolve into alveolar buds (AB) and eventually into terminal ducts (TD); this occurs from 40–60 days of age, with maximum developmental activity at ages 40–46 days [19]. The alveolar buds evolve into lobules of type 1 which are more highly differentiated structures than TEBs, TDs, or ABs, but do not produce milk. If the rat remains virgin, the mammary tissue will remain in this stage. With pregnancy, or a dramatic increase in prolactin, the type 1 lobules differentiate further into type 2 then type 3, the latter of which produce milk. There is a sharp decrease in TEB concentration after age 55 days. This progression of development differs somewhat depending on anatomic region of the gland; in the anterior regions, cervical and thoracic, the transition of TEB to TDs and AB is prolonged compared to the abdominal and inguinal regions [19].

Under normal conditions DMBA is most effective at producing tumors during the most active transition period of TEB evolution into AB [19]. There is a sharp decrease in tumor yield if DMBA is given after age 55 days because proliferative activity of the mammary epithelial cells at risk has decreased. A paradigm in carcinogenesis is that mutation is required for malignant transformation. As the rate of cell division decreases, risk of malignant transformation decreases as well, all other factors being similar. The period of 50–55 days in the Sprague–Dawley rat is toward the end of the high cell cycling activity of the mammary tissue under normal conditions. DMBA is most often given at this time by toxicologists because the historically optimum dose of 20 mg yielded significant toxicity in younger animals. With lower doses, it is feasible to dose at younger ages.

Our interpretation of the results presented here is that LL treatment substantially accelerated mammary tissue differentiation and, due to the time course of rat mammary development, the DMBA was relatively less effective at age 52 days in the LL group because the tissue had differentiated beyond the optimum period of carcinogen sensitivity. Our results are consistent with the idea that a greater tumor yield occurred in the mammary tissue of LD animals wherein the TEB to AB transition in the mammary tissue was occurring in the normal window of DMBA sensitivity [19]. On the other hand, the window of sensitivity was missed in the LL animals, where mammary gland differentiation had developed beyond the time of susceptibility to the DMBA (at 52 days of age). The fact that 60% of the LD rats had a tumor in the abdominal and/or inguinal regions, whereas only 11% of the LL group had a tumor in the same region, and the fact that the abdominal/inguinal regions are expected to have a more limited period of TEB differentiation support this interpretation. The appearance of lobular cysts in the LL group is also in agreement with this interpretation.

The results of this study are inconsistent with the results reported by Shah et al. [14], in which constant light resulted in increased tumor yield. They reported a greater concentration of TEBs at 55 days of age in rats exposed to LL and cited this as the explanation for the greater tumor yield by DMBA delivered at age 55 days. Their results must be considered in the context of the hypothesis that elevated exposure to estrogen in utero will increase lifetime risk of breast cancer [20] by increasing mammary gland mass from the begin-

ning of life. Indeed, elevated in utero estrogen has been shown to lead to altered mammary gland development and precocious puberty. Hilakivi-Clarke et al. [21] tested the hypothesis that feeding pregnant rats a diet high in n-6 polyunsaturated fatty acid (PUFA) would significantly raise circulating 17β-estradiol levels, and would result in increased DMBA-induced mammary tumor yield in their female pup offspring during their adulthood. The results confirmed their prediction wherein the rats exposed in utero to high PUFA, and higher in utero estrogen exposure, not only experienced earlier onset of puberty, but also that their mammary glands contained significantly higher numbers of the epithelial structures as targets for malignant transformation. It is probable that exposure of the rat dams to light in the Shah et al. studies resulted in increased estrogen (in addition to the reported increase in prolactin) and therefore not only accelerated mammary gland development in the pups, but also increased the amount of breast tissue. The Hilakivi-Clarke et al. [21] and Shah et al. [14] results taken together suggest that the constant light exposure of dams while pregnant resulted in a greater mammary tissue mass at the beginning of life. In Hilakivi-Clarke et al., rats with high PUFA in utero and high in utero estradiol exposure did not have elevated estradiol at 4 weeks of age but did have a significantly elevated fat pad area and epithelial density.

Differences between the study reported here and the experiments of Shah et al. [14] include differing levels of administered DMBA: 10 mg/100 g body wt. (~17 mg per rat) vs. 4.7 mg/100g body wt. (8 mg per rat) in our study. In addition, Shah used a different strain of rats: Holtzman vs. Sprague–Dawley. However, as noted above, the most important difference may be related to the timing of LL exposure. Shah et al. exposed the dams from conception and the female pups continuously from birth. Whereas, in the study reported here, constant light exposure began at 26 days of age, presumably inducing earlier TEB–AB conversion, but with no increase in levels of TAB-AB, thus taking the timing of TAB–AB conversion out of a window of sensitivity to DMBA and yielding lower tumor development activity at 52 days of age, compared with the LD rats.

The rat mammary model has yielded valuable insight into the pathogenesis of breast carcinoma over the past 40 years [22]. There are, however, important differences in mammary development and tumorigenesis between rat and human. Normally age is closely related to mammary gland developmental stage and structure concentrations in the rat: it is less clearly related in the human. In the rat, carcinomas arise primarily in TEBs and TDs, and more advanced structures such as lobules can give rise to cysts. In humans, most mammary tumors appear to arise in the terminal ductal lobule unit (TDLU); these structures develop early and persist into the post-menopausal years [23]. While there appears to be a 'window of susceptibility' to carcinogen in the rat, there are no data showing this in humans [23]. Therefore, advanced mammary development and elevated hormones in women due to chronic light exposure at night and altered daytime lighting might increase lifetime risk of breast cancer from either spontaneous mechanisms or specific toxic exposures.

This work represents an initial effort to address age-specific mammary tissue development as influenced by timing of light exposure. An interesting aspect of this study is that the light exposure was given from immediately prior to onset of puberty, as opposed to light exposure in utero through development. We intend to further investigate the importance of light exposure on mammary gland development, both in timing and in spectral content. The corresponding changes in hormone levels with various lighting regimens and conditions will also be investigated. By determining the influence of patterns of light exposure on mammary tissue development we expect to gain insight into mammary gland tumorigenesis as well.

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