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Exposure to light-at-night increases the growth of DMBA-induced mammary adenocarcinomas in rats

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Abstract

In order to assess whether light exposure at night influences the growth of mammary tumors, as well as the role of melatonin in this process, female rats bearing DMBA-induced mammary adenocarcinomas were exposed to different lighting environments. Animals exposed to light-at-night, especially those under a constant dim light during the darkness phase, showed: (a) significantly higher rates of tumor growth as well as lower survival than controls, (b) higher concentration of serum estradiol, and (c) lower nocturnal excretion of 6-sulfatoxymelatonin, without there being differences between nocturnal and diurnal levels. These results suggest that circadian and endocrine disruption induced by light pollution, could induce the growth of mammary tumors.

Keywords: Mammary cancer; Light; 6-Sulfatoxymelatonin; DMBA; Melatonin; Estradiol

1. Introduction

The exposure to light-at-night may be a factor which increases the risk of breast cancer. The basis of this hypothesis is that light during the dark phase of a light/dark cycle suppresses the pineal production of melatonin [1], which, in turn, could represent a relative increase in the synthesis of estrogens by gonads as well as a 'circadian disruption' [2,3]. This suggestive hypothesis has been mostly studied in humans, with epidemiologic approaches. Thus, the low incidence of breast cancer among blind women [3–7], as well as the inverse association between breast cancer incidence and degree of visual impairment [8], are explained by the total or partial suppression of the light input which could mediate an increase in melatonin circulating levels, responsible for the low incidence of tumors. On the contrary, the high incidence of breast cancer among women exposed to light during night, such as shift workers [9–12], or exposed to low-frequency electromagnetic fields [13,14] could be explained by the decreased melatonin synthesis under these environmental conditions.

The objective of this work was to assess whether light exposure at night influenced the rate of growth of

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mammary adenocarcinomas as well as the role of melatonin in these changes.

2. Materials and methods

2.1. Animals, lighting conditions and experimental protocol

Female Sprague–Dawley rats, 55 days of age, received single intragastric doses of dimethylbenzanthracene (DMBA) (20 mg in 1 ml of sesame oil). From that moment on, the animals were examined on a weekly basis in order to detect the appearance of mammary tumors. When mammary tumors of 1 cm diameter were palpated, the animals were assigned to different experimental groups (16 rats per group) defined by specific patterns of illumination (see Fig. 1): *Group LD*, the animals were maintained under a 12 h light (300 lux)/12 h darkness photoperiod; *Group LL*, the rats were placed under constant lighting (300 lux); *Group* LD_{PLE}, the same as LD but applying light (30 min, 300 lux) half-way through the period of darkness (PLE = partial light exposure); *Group* LD_{CDLE}, the same as LD, but maintaining a dim light (0.21 lux) present throughout the period of darkness (CDLE = constant dim light exposure). Light was provided by ceiling-mounted daylight fluorescent tubes controlled by electronic timers. Dim light nocturnal exposure was provided by incandescent bulbs (15 w) which remained on all the time. Light intensity was measured at the level of the cages with a digital photometer (Gossen Mavolux, Elangen, Germany) and the cages were placed in a circle equidistant from the light source, on a horizontal surface, so that each receive the same amount of light. In all cases, the evolution of the tumors (size and number), as well as the survival of the animals, was recorded. The size of the tumors was calculated as in Rose and Noonan [15]. There was also a daily analysis of the estrous cycle in all rats by inspection of the vaginal smear. After 12 weeks, animals were individually placed in metabolic cages and the urine of a 24 h period was collected in two 12 h separate fractions corresponding to the light (day) and darkness (night) periods. Urine samples from the animals exposed to constant light (LL) were also collected during a 24 h period divided into subjective day and subjective night, defined by the locomotor activity (night period) detected by actimeters. Urine samples were frozen until used for 6-sulfatoxymelatonin determination (RIA kits from Stockgrand Ltd, Guildford, UK). Two days later the animals were sacrificed and blood samples collected for determination of serum estradiol (ELISA kits from Diametra S.r.l., Italy).

2.2. Statistical analysis

Body weight, tumor surface and number of tumors, 6-sulfatoxymelatonin concentration in urine, and serum estradiol concentrations, were analyzed by one-way ANOVA followed by the Student–Newman–Keuls multiple comparisons test. Survival data were analyzed by the Kaplan–Meier method, using the Log Rank and the Breslow statistic tests for comparing survival curves. Day–night differences in 6-sulfatoxymelatonin excretion were analyzed by Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

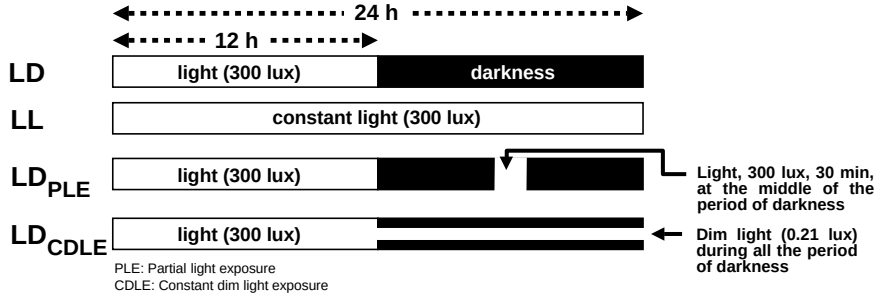

Fig. 1. Diagram showing the patterns of illumination for each experimental group.

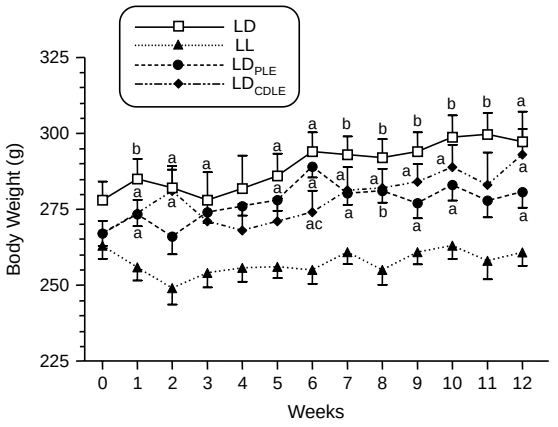

Fig. 2. Effects of different patterns of illumination on body weight of rats with DMBA-induced mammary tumors. Values are means ± SEM (a) $P<0.05$ vs LL; (b) $P<0.001$ vs LL; (c) $P<0.05$ vs LD.

3. Results

3.1. Effects of the exposure to light-at-night on body weight

The body weight of animals exposed to constant light (LL) was significantly lower than those of controls (LD) or those animals subjected to light contamination of the darkness period (LD_{PLE} or LD_{CDLE}); the latter also showed body weights lower than controls (LD), although differences were not significant (Fig. 2).

3.2. Effects of the exposure to light-at-night on tumor size, number of tumor and survival probability

Fig. 3 shows the evolution of tumors size depending on the pattern of illumination to which the animals were subjected. The lowest rate of tumor growth corresponded to rats under LD. Animals exposed to LL, LD_{PLE} or LD_{CDLE} showed significantly higher rates of tumor growth than animals under LD photoperiod. From the 7th week of exposure to nocturnal light pollution, the average tumor size in those rats in LD_{CDLE} was significantly larger than in the animals under LD photoperiod, whereas it took 9 weeks for animals in LD_{PLE} and 11 weeks for those under constant light (LL), to develop mammary tumors significantly bigger than rats in LD. After 12 weeks of experiment, no differences in tumor size were found among the animals exposed to light at night, depending on the pattern of lighting (LL, LD_{PLE} or LD_{CDLE}). We did not find significant differences in the number of tumors among the animals of the four experimental groups.

Animals exposed to constant dim light at darkness (LD_{CDLE}) had the lowest survival probability ($P<0.01$), and after 12 weeks of experiment only 50% of the animals were alive. There were no significant differences in terms of survival between controls (LD) and rats exposed to LL or LD_{PLE} (Fig. 4).

3.3. Effects of the exposure to light-at-night on urinary excretion of 6-sulfatoxymelatonin

Excretion of the melatonin metabolite 6-sulfatoxymelatonin reflects pineal synthesis and

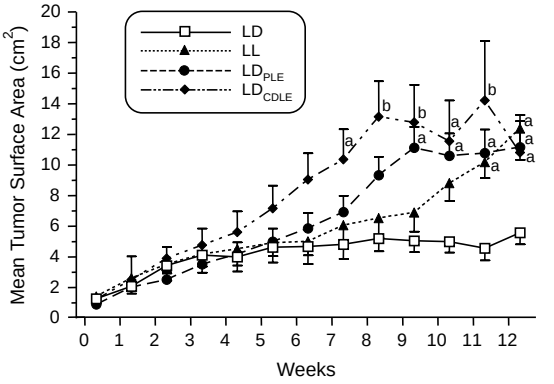

Fig. 3. Time course of changes in tumor size in animals with DMBA-induced mammary tumors, depending on the different patterns of illumination. (a) $P<0.05$ vs LD; (b) $P<0.01$ vs LD.

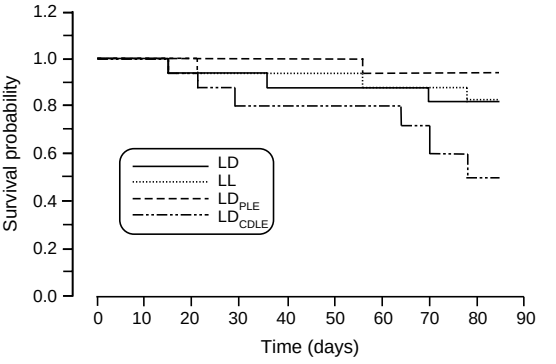

Fig. 4. Kaplan–Meier survival curves for the different experimental groups.

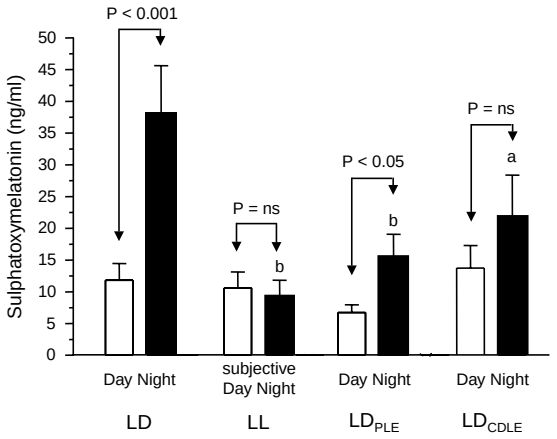

Fig. 5. Excretion of the melatonin metabolite 6-sulfatoxymelatonin in animals with DMBA-induced mammary tumors after 12 weeks of exposure to different patterns of lighting. Urine was collected in two 12 h separate fractions corresponding to the light (day) and darkness (night) periods (see Section 2). Data are expressed as mean ± SEM (a) $P < 0.05$ vs LD; (b) $P < 0.001$ vs LD.

secretion of the hormone. As expected, those animals under a light/darkness cycle (LD) show a clear difference between the excretion of 6-sulfatoxymelatonin during the light and the darkness stages (Fig. 5). The effects of light exposure during darkness on melatonin synthesis were different depending on the pattern of light pollution applied. Thus, the constant exposure to dim light during darkness (LD_{CDLE}) decreased the nocturnal excretion of 6-sulfatoxymelatonin and abolished the day–night rhythm; similar effects were observed in rats in constant light (LL); the application of light (300 lux, 30 min) half-way through the period of darkness also decreased the nocturnal excretion, but preserved the diurnal rhythm, there being a higher excretion of 6-sulfatoxymelatonin during night than during day.

3.4. Effects of the exposure to light-at-night on estrous cycle and serum estradiol concentration

While the rats placed in cyclic light (LD) showed 4–5 day-length estrous cycles, those rats exposed to constant light (LL) showed the characteristic persistent vaginal estrous. Out of the animals subjected to light-at-night, those which underwent constant, although low-intensity, light exposure (LD_{CDLE}) showed persistent vaginal cornification in a similar way to rats in LL; however, when nocturnal light contamination was partial (LD_{PLE}), some animals (4 out of 13) continued to cycle, although not in a regular 4–5 day way, while most were in estrous. Serum estradiol concentration was measured in blood samples obtained on sacrificing the animals; on that day, 40% of the animals from the LD group were in a proestrous or estrous phase, whereas 60% were in a diestrous or metaestrous one; all the animals from LL, LD_{PLE} or LD_{CDLE} groups showed vaginal cornification typical of persistent estrous. Despite the great dispersion of data, animals exposed to constant light exposure at night (LD_{CLE}) showed serum concentrations of estradiol significantly higher than controls (LD), and even higher than the rats under constant light (LL) or partial light contamination at night (LD_{PLE}) (Fig. 6).

4. Discussion

The increase of breast cancer risk in industrialized countries has been attributed, among other multiple factors, to 'light pollution' during night [16]. Since the development and generalization of electriclighting, our nocturnal environment is 'contaminated' by light of enough intensity as to influence our circadian system [17]. Melatonin is a pineal hormone which is synthesized and released into the blood only under

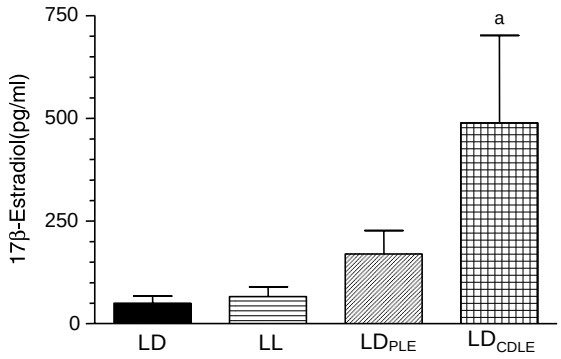

Fig. 6. Effects of different patterns of illumination on serum estradiol level of rats with DMBA-induced mammary tumors. Data are expressed as mean ± SEM (a) $P < 0.05$ vs LD.

environmental darkness; light inhibits hormone synthesis and the light/dark daily rhythm synchronizes the normal secretion pattern [1,18]. Melatonin has been demonstrated to act as an antiestrogenic factor, which in vivo as well in vitro, prevents the induction, progression and metastasis of hormone-dependent adenocarcinomas [19–22]. Taken together, all the arguments expounded above support the hypothesis of a possible relationship between light-at-night and breast cancer [2,16]. The epidemiological approaches to this hypothesis in humans have been exposed in the introduction of this article [2,4–14] and point to a relationship between breast cancer risk and light input. While blindness or any other factor that reduces the input of light into the circadian system, probably as a result of a relative increase in the production of melatonin, decreases the risk of mammary carcinogenesis [4,6–8], the disruption of the normal pattern of melatonin secretion because of light at night (such as in shift workers) has the opposite effects [5,9–14].

Most experimental studies have focused on the effects of light in the initiation of the tumors rather than on the evolution of preexistent tumors. Constant light reduces the latency and increases the number of DMBA-induced mammary tumors in rats [23], as well as increasing the incidence of different spontaneous tumors in female CBA mice and reducing their life span [24]. Light exposure at night also increases the growth of different kinds of transplantable tumors in rats [25–28].

We have herein demonstrated that the growth of DMBA induced mammary adenocarcinomas is enhanced by exposing rats to light during the darkness period. If we compare the effects of our three patterns of light exposure on tumor growth, the constant dim light exposure at darkness (LD_{CDLE}) seems to be the greatest tumor growth-inducer. It is at least under this lighting pattern that the increase in tumor size in relation to the controls comes earlier and the mortality rate is higher. Even considering tumor size at the end of the experiment (12 weeks), when there are no differences between the three lighting conditions, it is important to consider that results are expressed as mean tumor size of the living animals in each group, and many animals of the LD_{CDLE} group, those with the biggest tumors, died before the 12th week of the experiment. The strong effects of constant dim light contamination on tumor growth could depend on the decrease of melatonin production, absence of melatonin rhythm and the consequent increase of serum estradiol concentration observed in these animals. It is remarkable how low-intensity light exposure during the dark phase has similar effects to constant light in blocking melatonin secretion and stimulation of mammary tumor growth, results which agree with previous data in experiments with transplantable hepatoma cells [25,26]. The three light-treatments assayed (LL, LD_{PLE}, and LD_{CDLE}) induced significant decreases in the urinary excretion of 6-sulfatoxymelatonin (which reflect the melatonin secretion), although in animals under LD_{PLE}, because of the time of application of the light pulse, the rhythm of melatonin secretion was not fully abolished but rather its amplitude made smaller. Whereas exposure to dim light at night (LD_{CDLE}) or to constant bright light (LL) causes similar effects on melatonin secretion, serum estradiol concentration, tumor growth and animal mortality were higher under dim light exposure at darkness. The explanation for this fact is not clear and several hypotheses could be considered. One is the possible different effects on these two lighting patterns of prolactin (PRL) secretion, a pituitary hormone which influences the growth of DMBA-induced mammary tumors [15]. It is well known that the length of exposure to constant light modifies the control of PRL secretion by increasing the response to estradiol [29], whereas the effects of dim light at night on PRL secretion are unknown. The second hypothesis should consider the different metabolic effects of both light-treatments. Although several authors described no changes in body weight in rats [25,26] or mice [24] under LL, we found a significant decrease in body weight in animals under constant lighting but not in those exposed to dim light at darkness (LD_{CDLC}). These metabolic changes induced by LL could influence the evolution of the tumor in a different way than in animals under LD_{CDLC}.

Our results give experimental support to the epidemiological data describing a possible influence of nocturnal light in mammary carcinogenesis and encourage the study of melatonin-based treatments to reduce the risk of carcinogenesis in people exposed to light-at-night.

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