

Light during darkness, melatonin suppression and cancer progression

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Abstract

Over the past few years, we have shown that the surge of melatonin in the circulation during darkness represents a potent oncostatic signal to tissue-isolated rat hepatoma 7288CTC, which is an ER+ adenocarcinoma of the liver. This oncostatic effect occurs via a melatonin receptor-mediated suppression of tumor cAMP production that leads to a suppression of the tumor uptake of linoleic acid (LA), an essential fatty acid with substantial oncogenic properties. The ability of LA to promote cancer progression is accomplished by its intracellular metabolism to 13-hydroxyoctadecadienoic acid (13-HODE) which amplifies the activity of the epidermal growth factor receptor/mitogen-activated protein kinase pathway leading to cell proliferation. By blocking tumor LA uptake, melatonin effectively blocks the production of 13-HODE and thus, markedly attenuates tumor growth. A similar effect of melatonin is observed in tissue-isolated, ER+ MCF-7 human breast cancer xenografts and nitrosomethylurea (NMU)-induced rat mammary cancers. When male rats bearing tissue-isolated hepatomas are exposed either to constant bright light (300 lux) or dim light (0.25 lux) during the dark phase of a 12L:12D photoperiod, the latency to onset was significantly reduced while the growth of tumors was markedly increased over a 4 wk period as compared with control tumors in 12L:12D-exposed rats. In constant light- and dim light during darkness-exposed rats, melatonin levels were completely suppressed while tumor growth, LA uptake and 13-HODE production were markedly increased. Similar results were obtained in constant bright light-exposed female rats bearing tissue-isolated NMU-induced mammary cancers or MCF-7 human breast cancer xenografts. To date, these studies provide the most definitive experimental evidence that light exposure during darkness increases the risk of cancer progression via elimination of the nocturnal melatonin signal and its suppression of tumor LA uptake and metabolism to 13-HODE.

Introduction

Light introduced during dark phase literally “shuts-off” melatonin production – this is often referred to as the acute suppressive effect of light [1]. Nearly all studies on the effects of different wavelengths on hamsters, rats, and mice suggest that wavelengths in the blue and green portion of the visible spectrum have the strongest impact on circadian and neuroendocrine regulation particularly the suppression of nocturnal melatonin production [2]. Additionally, even low intensity light stimuli during the dark phase alter normal circadian function and suppress nighttime melatonin synthesis and secretion by the pineal gland. For example, dose-response (fluence-response) curves show that exposure to 1.2 lux (0.5 $\mu\text{W}/\text{cm}^2$) and 0.16 lux (0.074 $\mu\text{W}/\text{cm}^2$) of white light during the night is sufficient to suppress melatonin in rats and hamsters, respectively [3,4]. In fact, there is a wide range of sensitivity to light for melatonin suppression among different mammalian species [5].

Melatonin has emerged as an important, circadian neurohormonal regulator of neoplastic growth both at physiological and pharmacological levels, particularly in experimental murine and human breast cancer [6,7]. In nitrosomethylurea (NMU)- or dimethylbenzanthracene (DMBA)-induced models of rat mammary tumorigenesis, daily, late afternoon melatonin injections (a few hours before lights off) or melatonin in the drinking water, at pharmacological concentrations, substantially reduce mammary tumor incidence and multiplicity. Of particular importance is the fact that the endogenous, nocturnal melatonin signal itself serves as a physiological regulatory signal in the control of mammary carcinogenesis. Evidence for this comes from studies showing that either DMBA- or NMU-induced mammary tumorigenesis is enhanced in pinealectomized rats (i.e., no melatonin signal) as compared with non-pinealectomized with an intact nocturnal surge of melatonin. The tumor promoting effects of pinealectomy are prevented by melatonin injections [6,7]. Also, physiologically relevant concentrations of melatonin (i.e., nocturnal melatonin levels) directly inhibit the proliferation of estrogen receptor (ER)+ MCF-7 human breast cancer cells *in vitro* by delaying the transit of cells from G_1/G_0 to the S-phase of the cell cycle, thus prolonging the cell cycle, and by suppressing DNA synthesis. There is also good evidence that melatonin acts as a differentiating agent [8].

Experiments using constant bright light (400–800 lux) as a method of “physiological” pinealectomy have been employed as an alternative strategy for extinguishing the nocturnal melatonin signal to enhance mammary tumorigenesis in both the Sprague-Dawley rat DMBA- and NMU-induced model systems both of which are prolactin (PRL)- and E_2 -dependent [6,9–11]. Although it is tempting to ascribe enhanced mammary tumorigenesis in both pinealectomized and constant light-exposed animals to the lack of a nightly melatonin surge, it is important to note that these two manipulations are not exactly equivalent in the outcomes they produce. For example, while the circadian melatonin signal is eliminated in both pinealectomized and constant light-exposed animals, the circadian activity rhythm, which is still synchronized by

the in pinealectomized rats, initially free-runs in constant light-exposed rats with a periodicity of about 25.1 hours [12]. However, more prolonged exposure to constant lighting conditions (several weeks to months) culminates in complete suppression of circadian rhythmicity and its replacement by ultradian rhythms (i.e., periodicity of < 20 hours) [13].

Based on these studies, Stevens [14] postulated that the melatonin suppressive effects of “light-at-night” (LAN) in human populations may contribute to an increased risk of breast cancer. Indirect support for this hypothesis comes from recent epidemiological studies showing that blind individuals, who presumably do not perceive light, have a significantly lower risk of cancer, particularly breast cancer, as compared with individuals with normal vision [15]. In fact, in one of these reports, the degree of breast cancer risk was inversely related to the degree of visual impairment. Animal studies also show that carcinogen-induced mammary tumorigenesis is decreased in constant dark-exposed rats as compared with animals on diurnal lighting [11]. The implication of these findings is that in addition to alterations in melatonin rhythmicity that occur in response to blindness [16], blind individuals may be “protected” from the melatonin-suppressive and thus cancer-promoting effects of LAN. Conversely, the risk of breast cancer is increased up to 60% in women who work night shifts suggesting that exposure to LAN-induced melatonin suppression may be responsible for this elevated cancer risk [17–19]. We briefly review herein our published and unpublished preliminary work that provides the first experimental support for the Stevens’ hypothesis with respect to cancer growth progression in rats bearing tissue-isolated liver and mammary cancers as well as in rats bearing tissue-isolated human ER+ human breast cancer xenografts.

Effects of constant light and dim light during darkness on the growth and fatty acid metabolism of tissue-isolated rat hepatoma

The growth of a variety of tumors including experimental murine and human breast cancer xenografts and particularly rat hepatoma 7288CTC is markedly stimulated by the essential fatty acid LA [20]. In hepatoma 7288CTC, LA is metabolized by a lipoxygenase to 13-HODE which we have recently shown to be the mitogenic signal for LA-stimulated hepatoma growth [21]. 13-HODE amplifies the activity of the epidermal growth factor receptor (EGFR)/mitogen-activated protein kinase (MAPK) [22]. In this regard, recent evidence from our laboratory [23] shows that melatonin inhibits the growth of hepatoma 7288CTC by blocking LA uptake and 13-HODE production via a melatonin receptor-mediated suppression of cAMP; these results have also been extended to experimental murine and human breast cancer (Blask et al., unpublished results).

We initially reported that a marked suppression in nocturnal plasma melatonin levels and a stimulation of tissue-isolated hepatoma growth and LA uptake and metabolism to 13-HODE was associated with light contamination of one of our animal rooms, during the dark

phase of a 12L:12D photoperiod, with approximately 0.2 lux ($0.06 \mu\text{W}/\text{cm}^2$) of indirect, reflected fluorescent light leaking through the door jam from an adjacent room [24]. Based on these results, we subsequently tested the effects of carefully controlled direct illuminance with dim light (0.25 lux at cage level) from a dedicated light source present inside the animal room during the dark phase on the growth and fatty acid metabolism of tissue-isolated hepatoma 7228CTC and circulating melatonin levels in male Buffalo rats [25]. As a positive control, we also included tumor-bearing animals that were exposed to constant bright light (300 lux). Not only were melatonin levels suppressed to the same extent by constant light and dim light during darkness, but tumor latency to onset and growth were markedly accelerated as compared with that in the 12L:12D control group. Furthermore, tumor LA uptake and metabolism to 13-HODE as well as tumor fatty acid content were also substantially elevated resulting from exposure of tumor-bearing animals to constant light or dim light during darkness. It is important to note that extended exposure to constant light results in a general circadian desynchronization including suppression of melatonin production whereas exposure to dim light during darkness specifically suppresses melatonin production while leaving other circadian activity (i.e., feeding behavior) intact. This would strongly argue that light during darkness-induced melatonin suppression, rather than general circadian disruption, is responsible for accelerated tumor growth via an enhancement of tumor LA uptake and 13-HODE production.

Effects of constant light on the growth and fatty acid metabolism of tissue-isolated Nitrosomethylurea (NMU)-induced rat mammary cancer

We next determined whether the growth-stimulatory effects of constant light on hepatoma 7228CTC could be extended to a breast cancer model such as the NMU-induced rat mammary tumor. We have shown that melatonin inhibits the development and growth of these tumors [6] while pinealectomy [8] or constant light exposure [9–11] increases tumor development as compared with rats on a 12L:12D light:dark cycle. Therefore, we initiated the development of tumors in donor female Buffalo rats by injecting them with NMU (50 mg/kg i.p.) at 50 days of age. Recipient adult female Buffalo rats that also had been maintained for two weeks on 12L:12D were randomized to either 12L:12D or constant light (L:L) one week prior to tumor transplantation. Following a number of passages, NMU-tumor tissue from donor rats was transplanted, in a tissue-isolated manner, into recipient female rats one-week following their placement into their respective photoperiods.

The mean (\pm SD) latency to onset of palpable tumors was 37.5 ± 5.6 days in the L:D group and 20.5 ± 2.7 in the combined L:L groups ($p < 0.05$). The growth of NMU-induced mammary tumors is markedly stimulated by exposure to L:L as compared with tumor growth under diurnal lighting conditions. Upon closer examination of the growth curves of the L:L group, it became apparent that we could further stratify this group into three sub-

groups each with different growth rates; however, the tumor growth rate in each subgroup was greater than in L:D rats. The tumor growth rates in two of the L:L subgroups were 5- to 6-fold higher whereas tumors grew twice as fast in the third L:L subgroup as compared with L:D controls [Blask et al., unpublished results].

In the L:L group, LA uptake and 13-HODE production were significantly higher than in the L:D group. The much lower production of 13-HODE by NMU-induced mammary tumors as compared with hepatoma 7228CTC may explain the markedly lower growth rate of these tumors. These results extend our observations on the effects of constant light on the growth and LA metabolism of hepatoma 7228CTC to the tissue-isolated NMU mammary tumor model and provide the first evidence that elimination of the melatonin signal by constant light exposure stimulates mammary tumor growth via a stimulation of LA uptake and metabolism. Moreover, the perfusion of a tissue-isolated NMU-induced rat mammary tumor with a physiological concentration of melatonin (1 nM) completely blocks total FA and LA uptake as well as 13-HODE production providing further evidence that elimination of the physiological melatonin signal would result in a disinhibition of LA uptake and metabolism to 13-HODE and consequently tumor growth [Blask et al., unpublished results].

Effects of constant light on the growth and fatty acid metabolism of tissue-isolated human MCF-7 breast cancer xenografts

We next wanted to test whether constant light suppression of the nocturnal melatonin signal would result in a stimulation of human breast cancer growth. In order to do this, we implanted xenografts of MCF-7 human breast tumors into adult female nude rats in a tissue-isolated manner as we had done in the case of rat hepatoma 7228CTC and NMU-induced rat mammary adenocarcinomas. The MCF-7 breast cancer xenografts were obtained from donor female nude mice implanted with 90-day release estradiol pellets (2.5 mg) that had developed solid tumors as a result of being inoculated s.c. in the right axilla with MCF-7 human breast cancer cells ($\approx 10^7$ cells/mouse) that had been grown in culture in DMEM containing 10% FBS. Rats were maintained on 12L:12D prior to tumor implantation and until 40 days following implantation at which time the mean tumor weight was 2.5 g. At this time 4 tumor-bearing rats were switched to a photoperiod of 24L:0D while the remaining 6 animals were maintained on a 12L:12D photoperiod.

Following their transfer to constant light, MCF-7 human breast cancers grew at a rate 7-fold higher than control tumors in animals maintained on 12L:12D. Moreover, in the L:L group, LA uptake and 13-HODE production were significantly higher ($p < 0.05$) than in the L:D group. These results extend our observations on the effects of constant light on the growth and LA metabolism of tissue-isolated hepatoma 7228CTC and NMU mammary tumors to a human breast cancer model and provide the first evidence that elimination of the melatonin signal by constant light exposure stimulates mammary tumor growth via a stimulation of LA uptake

and metabolism to 13-HODE and consequently tumor growth [Blask et al., unpublished results]. One might question whether the physiological melatonin signal in the nude rat has any relevance to the melatonin signal in humans. We determined that the female nude rat does indeed exhibit a circadian melatonin signal that is quite similar to the physiological melatonin signal in women in terms of the timing, duration and particularly the amplitude [26]. Therefore, the amount of melatonin to which MCF-7 breast cancers were exposed under 12L:12D in our preliminary experiment were virtually identical to what human breast cancers would be exposed to in humans. These data are congruent with our previous studies [6] showing that the exposure of MCF-7 cells in culture to physiological levels of melatonin causes a 60% to 70% inhibition of cell growth which is reversible with the removal of melatonin from the culture medium. Additionally, the perfusion of an MCF-7 tumor from an L:L exposed nude rat with a physiological level of melatonin completely blocks total FA and LA uptake as well as 13-HODE production by the tumor [Blask et al., unpublished results].

Other preliminary studies [Blask et al., unpublished results] from our laboratory show that the perfusion of a tissue-isolated MCF-7 xenograft with physiological melatonin inhibited LA uptake and 13-HODE release as expected and also caused a 36% decrease in tumor DNA content and a 76% reduction in [³H]-thymidine incorporation into DNA. However, coperfusion of a tumor with melatonin+13-HODE blocked the inhibitory effect of melatonin and increased DNA content and [³H]-thymidine incorporation by 60% over the control tumor; melatonin inhibition of LA uptake was unaffected by simultaneous 13-HODE perfusion. Perfusion of a control tumor with 13-HODE alone, at levels that yielded a release rate equivalent to that in constant light, also increased tumor DNA content and [³H]-thymidine incorporation by over 40% as compared with the control tumor. As in the case of tissue-isolated hepatoma and NMU-induced mammary tumors, these results show that human ER+ breast cancer is sensitive to the inhibitory actions of physiological melatonin and provide further support for the hypothesis that the suppression or complete elimination of the physiological melatonin signal by LAN would result in a disinhibition of LA uptake and metabolism to 13-HODE and consequently the growth of human breast cancer *in vivo*.

Final commentary

The published and unpublished studies briefly reviewed herein provide the first experimental evidence that directly supports the Stevens' hypothesis [14] and extends it to another cancer type (i.e. liver cancer) with respect to increased risk of growth progression. Our experimental approach does not address whether melatonin suppression by LAN increases the risk of *de novo* development of breast or any other cancer. However, our preliminary results also demonstrate, for the first time, that exposure of rats bearing ER+ human breast cancer xenografts to constant illumination results in accelerated tumor growth ostensibly due to a suppression of mela-

tonin production. Since prolonged exposure to constant light mitigates desynchronization in general circadian activity [12,13], it cannot be ruled out that general circadian disruption, rather than, or in addition to a specific suppression of melatonin production, is the stimulus for breast cancer growth progression in our model systems for breast cancer growth progression. Nevertheless, our experiments with rat hepatoma and dim light during darkness strongly indicate that a specific LAN-induced suppression of melatonin increases cancer progression [24,25]. Only through additional experimentation will we be able to determine whether the risk for the development and/or growth progression of any malignant neoplasm, responsive to the nocturnal melatonin inhibitory growth signal, is increased by LAN-induced melatonin suppression.

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