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An Overview of Melatonin and Breast Cancer [2]

Exploring melatonin's unique effects on breast cancer cells

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It is well established that melatonin has oncostatic effects both in vitro and in vivo on various types of cancers, with a unique effect on breast cancer cells. In breast cancer, melatonin is capable of disrupting estrogen-mediated cellular pathways, resulting in a net reduction in estrogenic stimulation of cells. The pineal production of melatonin follows a diurnal rhythm, with peak production at about 2 a.m. Studies have shown that circadian disruption, specifically night shift work, is correlated with an increased risk of developing breast cancer. Since excessive exposure to estrogen is a well-established risk factor for breast cancer development, some researchers hypothesize that the increased risk in night shift workers is due to a relative increase in estrogenic stimulation when melatonin production is disrupted. Several prospective studies have demonstrated this inverse correlation between melatonin metabolites and incident risk of developing breast cancer. Further supporting this correlation is observational data that shows melatonin levels are lower in women with an established diagnosis of breast cancer. In addition to proper light/dark exposure, oral supplementation of melatonin may have benefit in women who are at risk of developing breast cancer, as well as those with an established diagnosis. Therapeutic supplementation is based on the causal link between low melatonin levels and breast cancer development, the diverse mechanisms of its oncostatic effects, and the clinical data demonstrating its benefit in patients with established breast cancer.

Melatonin (N-acetyl-5-methoxytryptamine) is an indolamine that is produced from the amino acid L-tryptophan within the pineal gland as well as in many extrapineal tissues, including the gastrointestinal tract and lymphocytes. Melatonin production in the pineal gland is ultimately under the control of the light/dark cycle through the retinal-suprachiasmic nucleus, with peak production during the dark phase of an approximate 24-hour cycle. Increased melatonin production during darkness is due to induction of the rate-limiting enzyme in its synthesis of arylalkylamine N-acetyltransferase (AA-NAT).[1] This enzyme is not induced when light is present on the retina, therefore light at night (LAN) attenuates melatonin production.

In 1978, when the role of lifetime excess estrogen exposure was gaining widespread recognition as a risk factor for breast cancer, Cohen, et al, proposed that diminished function of the pineal gland may increase risk of breast cancer by increasing exposure to circulating estrogens.[2] He based this hypothesis on several observations: The incidence of breast cancer is highest in countries in which pineal calcification is highest; patients taking chlorpromazine, a drug that raises melatonin, have lower rates of breast cancer; in vitro data suggests melatonin may have direct effects on breast cancer cells; and melatonin receptors were discovered on human ovarian cells, which suggests melatonin may have a direct influence on ovarian production of estrogen. In 1987, based on Cohen's work, Richard Stevens formed the predictive hypothesis that women who are exposed to LAN will have higher rates of breast cancer.[3]

Rodent studies have confirmed that circadian disruption or pinealectomy can lead to spontaneous tumor development and increased growth and metastatic potential of existing tumors.[4,5] This, along with epidemiological evidence of higher cancer rates in night shift workers, has led the World Health Organization's International Agency for Research on Cancer to deem night shift work, "probably carcinogenic to humans (Group2A)."[6] Further support for LAN's influence on breast cancer specifically can be found in a 2008 landmark study that used data from the US Defense Meteorological Satellite Program to compare LAN and breast cancer incidence in 147 communities in Israel. The communities with the highest LAN had a 73% higher rate of breast cancer compared to the darkest communities.[7]

While studies on pinealectomized rodents and those exposed to LAN suggest that reduced melatonin has a causal role in the growth and development of tumors, it would be inaccurate to imply that suppression of melatonin is the only known biological reaction to LAN. The biological effects of the light/dark cycle on the suprachiasmatic nucleus go beyond controlling melatonin production. Clock genes are also regulated by the light/dark cycle, and clock genes control cell cycle regulatory genes as well as genes involved in apoptosis.[8] These clock genes act independently from melatonin's actions.[9] For further information on clock genes, the reader is referred to a paper by R.G. Stevens, titled Circadian Disruption and Breast Cancer: From Melatonin to Clock Genes.[10]

Mechanisms of Actions

Melatonin is a lipophilic compound that is capable of binding to cell surface receptors (ML1, ML2), cytosolic sites (calmodulin), and directly to nuclear DNA binding sites (nuclear receptors RZR/RORα). Overall its effect on cancer cells is oncostatic at physiological levels, or cytotoxic at higher concentrations. Its direct antioxidant, antimitotic, antiestrogenic, prodifferentiating and antimetastatic effects have been well characterized. In addition to direct cellular actions, melatonin influences the hypothalamic-pituitary axis (HPA) and immune system, which confers anticancer effects through modulation of hormonal and immune-mediated pathways of proliferation/metastasis.

Perhaps the best characterized oncostatic effect of melatonin results from the net reduction of estrogenic stimulation to breast cancer cells.[11] Estrogens are involved in many aspects of the malignant process, including proliferation, angiogenesis, metastasis, immune evasion, and immortality. Melatonin has demonstrated attenuation of all of processes in the presence of estrogen. Systemically, melatonin affects the hypothalamic-pituitary-ovarian (HP) axis, which results in lower circulating levels of estrogen and progesterone.[12] On a cellular level, melatonin acts as a selective estrogen receptor modulator (SERM) through decreased expression of estrogen receptor alpha and reduction in the ability of estrogen-estrogen receptor alpha (ERα) complex to bind to the estrogen response element (ERE) on DNA. Melatonin also acts as a selective estrogen enzyme modulator (SEEM), reducing the activity of aromatase in cells, the enzyme responsible for conversion of androgenic precursors to estrogens.

Melatonin has demonstrated antagonist effects on E2-induced-ERα-mediated transcription of proliferative genes through its binding to calmodulin in the cytosol. When the ERα-calmodulin complex is bound by melatonin, a conformation change in the structure renders it incapable of binding to the promoter regions or the ERE of DNA. This reduces the transcription of many downstream genes that increase proliferation.[11]

Melatonin has been shown to decrease the motility and invasive capabilities of breast cancer cells (MCF-7) cells in vitro. This is partly due to melatonin's effects on cell surface adhesion molecules

such as E-cadherin and β1-integrin. These adhesion molecules allow for attachment of the cells within the extracellular matrix as well as to each other. These adhesion molecules are down-regulated by estrogen, increasing the invasive potential of the cell. Melatonin has been shown to increase the expression of these adhesion molecules in MCF-7 cells. In one study, melatonin at physiological doses (1nM), clearly lessened the invasive and metastatic effects of estradiol on MCF-7 cells.[13]

Intracellular actions independent of estrogen-signaling pathways continue to be elucidated as well. Melatonin is a potent antioxidant, reducing reactive oxygen species with a resultant decrease in DNA damage. It has also been shown to inhibit the activity of telomerase, the enzyme responsible for conferring immortality to cells, both in vitro and in vivo.[14] Another cytostatic effect of melatonin occurs through binding of one of its membrane receptors, ML1 and/or ML2, leading to a decrease in cellular uptake of linoleic acid. Reducing availability of linoleic acid results in an antiproliferative effect through the resultant decrease in its metabolite 13-hydroxyoctadecadienoic acid (13-HODE), which serves as an energy source and growth-signaling molecule for proliferative pathways such as epidermal growth factor receptor (EGFR)/mitogen-activated protein kinase (MAPK) pathway.[15]

Melatonin is capable of binding directly to the RZR/RORα steroidal region of DNA, which leads to altered transcription of several genes involved in cellular proliferation, such as tumor suppressor gene p21 and pro-inflammatory 5-LOX.[16] Separately, melatonin has been shown to potentiate the proapoptotic effect of retinoic acid, possibly through an interplay of the two molecules at the RZR/RORα region.[17]

Melatonin has also been shown to modulate immune function. One study showed extrapineal production of melatonin by lymphocytes increased the activity of interleukin-2 (IL-2) and IL-2 receptor system.[18] Clinically, many trials have shown a synergistic effect with therapeutic IL-2 administration in various cancers.[19] More generally, the systemic anti-inflammatory effects, as demonstrated in lower circulating levels of IL-6 and erythrocyte sedimentation rate (ESR) in patients taking melatonin, may affect both tumorogenesis and proliferative and metastatic pathways that are otherwise stimulated by inflammatory cytokines.[20]

Epidemiological Data

Night-Shift Work and Increased Risk of Breast Cancer
To date, 6 of 8 epidemiological studies have confirmed an association between night-shift work and increased risk of breast cancer. Taken together, a 2009 meta-analysis by Viswanathan and Schernhammer concluded that there is a 40% aggregate increase in relative risk (RR) of developing breast cancer in women working night shifts (95% CI, RR= 1.19–1.65).[21] Of note, confounding the meta-analysis is the lack of standardization in the definition of what constituted “night-shift work” between studies. Despite the lack of a unifying definition, an increased risk of breast cancer with the increasing duration in years of working “night shifts” was consistent across studies.

Two of the most well-controlled studies assessed in the meta-analysis were prospective cohorts within the Nurses’ Health Study (NHS) and Nurses’ Health Study II (NHS II). In 1988, the ongoing NHS asked 78,562 women how many years total they had worked at least 3 nights per month. The answer was divided into 7 categories: 1–2, 3–5, 6–9, 10–14, 15–19, 20–29 or more than 30 years. In the ensuing 10 years, 2,441 women went on to develop breast cancer. While there was a small but measurable increase in risk of breast cancer in the groups working rotating shifts for 1–14 years (RR=1.08, 95% CI .90–1.18) and 15–29 years (RR=1.08, 95% CI .99–1.30), the relative risk (RR) for women who worked more than 30 years of rotating shifts was a statistically significant 36%

increase (RR= 1.36) (95% CI, RR=1.04–1.78).[22] In the other prospective cohort study, the NHS II, 115,022 women were followed and 1,352 developed cancer. Using the same definition and categories as above, those women who had worked over 20 years of night shift work had a statistically significant increased risk, RR of 1.79 (95% CI, RR= 1.06–3.01). Both of these studies were well controlled for confounders such as body mass index, reproductive history, family history, smoking status, age, use of hormones, and benign breast disease.[23]

In a meta-analysis published in 2005, 6 of 7 flight attendant studies showed an increased risk of developing breast cancer. The authors concluded that the risk of developing breast cancer is increased by 44% for flight attendants versus controls from the general population. Of course, there are confounders that are unique to this population, such as time zone changes and radiation exposure. Nonetheless, these studies lend further support to the associated increased risk of work involving circadian disruption.[24]

Sleep Duration and Risk of Breast Cancer Development

While epidemiological data largely confirms the expected trend of increased risk of breast cancer in women with circadian disruption through working night shifts, prospective studies assessing sleep duration and associated risk are conflicting and inconclusive. In a prospective study of 12,222 women in Finland, 242 developed breast cancer during the study period. Women who reported getting more than nine hours of sleep per night had a 31% lower relative risk of developing breast cancer compared to those reporting 7–8 hours of sleep per night (RR 0.69 95% CI=0.45–1.06).[25] In another prospective study, a cohort of the NHS, there was no significant difference in the risk of developing breast cancer in those that slept more than 9 hours compared with those who slept less (OR 0.95 95% CI = 0.81–1.11).[26] One population-based case control study of 4,033 community-dwelling women with breast cancer compared to 5,314 controls had an unexpected, yet nonsignificant, increase in the risk of breast cancer in patients who reported more than 9 hours of sleep per night (RR 1.13, 95% CI 0.93–1.37).[27] A fairly large study of 23,995 Japanese women found a significantly higher risk in women who slept 6 hours per night or less (HR=1.62 95% CI 1.05–2.50).[28]

One explanation for these conflicting results is that unlike night-shift work, which has been shown to result in reduced melatonin production, a decrease in sleep duration may not necessarily correlate with melatonin levels. A 2009 review of studies looking at melatonin metabolites and the duration of sleep supports this. [29] It is also possible that melatonin levels in the sleeping subjects varied according to ambient light levels, a confounder not accounted for in any studies assessing sleep duration.

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Melatonin Metabolites and the Associated Risk of Developing Breast Cancer

Melatonin can be directly measured in the plasma, although this approach has fallen out of favor because it is unnecessarily invasive and requires sleeping in an observational facility. More recently, urinary metabolites, such as 6-sulphatoxymelatonin (aMT6s), have been used as a surrogate marker of melatonin. Urinary aMT6s correlates well with melatonin levels in blood and saliva and

has become the predominant means of measuring melatonin.30,31 There are several ways of measuring aMT6—as a 24-hour period urine collection, a timed overnight collection, or as a first morning void—the latter two correlating specifically with overnight melatonin secretion.[32,33]

Several prospective studies have considered the relationship between melatonin, as measured by its major urinary metabolite aMT6s, and incident risk of developing breast cancer. The first was the Guernsey III study, named for the location of Guernsey Island in the British Isles. The Guernsey III recruited 5,093 women between 1977 and 1985. A 24-hour urinary sample was obtained at the time of recruitment. In 2001, using public records and pathology reports, 127 cases of breast cancer were found and 353 case controls matched for age, menopausal status, day of menstrual cycle or number of years postmenopausal, and recruitment date. There were no statistically significant differences in the 24-hour urinary levels of aMT6 in those with breast cancer compared to the case-matched controls, regardless of premenopausal or postmenopausal status (OR 0.99 95% CI 0.58–1.70).[34] One critic of this study commented that a 24-hour pooled urinary sample does not take into account the dynamic circadian fluctuation of melatonin. He wrote, “Not determining the time of the melatonin nightly upswing, the nocturnal peak, the duration of nocturnal melatonin exposure, and the slope of its rise and fall is akin to averaging amplitude- and frequency-modulated radio signals and being surprised when no music issues from a radio receiver.”[35] Indeed, ensuing studies used urinary samples that more accurately reflected the nocturnal peak of circulating melatonin. The results of these studies, outlined below, support the inverse association between nocturnal melatonin levels and the risk of developing breast cancer.

The NHS II recruited 116,671 female nurses aged 25–42 beginning in 1989. Of these, 29,613 participants agreed to participate in a sub-study between the years 1996–1999 that included a first-morning urine sample and two blood samples (age of nurses at the time of these samples was 32–49). A prospective, case-control study of 147 incident breast cancer cases were evaluated between the time of urine collection and May 2001. Urinary metabolite (aMT6s) levels were divided into quartiles using the 291 matched controls. Women in the highest quartile of urinary aMT6s had a 41% reduction in their relative risk of invasive breast cancer compared to women in the lowest quartile (OR 0.59 [CI] = 0.36–0.97).[36] Of note, this author also investigated whether there is any statistical correlation between higher levels of aMT6s and estrogen-receptor status as was implied by an earlier, smaller study. No association between receptor status and aMT6 was found in this larger, prospective cohort study.

In Northern Italy between 1987 and 1992, 10,633 women enrolled in the Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) trial. Blood and 12-hour (7 p.m.–7 a.m.) urine samples were obtained at the time of enrollment. Of the 3,699 participants who were postmenopausal, there were 178 incident breast cancers occurring between the time of enrollment and December 31, 2003, according to the local cancer registry. These cases were matched to 710 controls. Statistical analysis showed a significantly lower risk of invasive breast cancer for women in the highest quartile of overnight aMT6s compared to the lowest quartile (OR=0.56, 95% CI 0.33-0.97). Interestingly, the association was even stronger for never or past smokers (OR= 0.38, 95% CI 0.20–0.74).[37]

A case-controlled, nested, prospective study on the association of urinary aMT6s and incident breast cancer from the NHS used first spot urine samples collected from 18,643 cancer-free women between March 2000 and December 2002. The concentration of aMT6s from 357 postmenopausal women who developed breast cancer between the time of the sample collection and May 31, 2006, was compared to 533 matched controls. Again, an increased amount of aMT6s was statistically associated with a lower risk of breast cancer. The odds ratio for the highest versus the lowest quartile was 0.62 (95% CI 0.41–0.95).[38] Taken together, the above trials support the predictive hypothesis that lower nighttime levels of melatonin confer an increased risk of developing breast cancer, and, correspondingly, higher levels of melatonin confer benefit.

Night-Shift Work and Increased Risk of Breast Cancer

In 1981, Bartsch and colleagues published the first study demonstrating that melatonin levels are diminished in patients with breast cancer.[39] Separately, in 1982, a small study of 10 women with stage-I or -II breast cancer showed that women with breast cancer had lower levels of peak plasma melatonin concentration than control subjects.[40] Later, in a controlled clinical trial of 35 breast cancer patients, Bartsch and colleagues found that peak nocturnal serum concentration of melatonin is 48% lower in the breast cancer patients compared to age-matched controls. Further, the reduction in peak amplitude of melatonin directly correlated with the size of the tumor, with increasing tumor size staged as T1, T2, and T3 having reductions in peak melatonin amplitude of 27%, 53%, and 73% respectively.[41] In a follow-up study published in 1997, Bartsch and colleagues confirmed that melatonin levels are significantly lower in patients with existing breast tumors. In this case-controlled study of 17 primary breast cancer patients awaiting surgery, urinary aMT6s was measured from 10 p.m. to 6 a.m. in all patients. The total excretion of aMT6s was 48% less in the breast cancer group compared to the 35 matched control subjects. Further, the researchers confirmed their earlier finding that there was a significant and inverse correlation of aMT6s with increasing tumor size in patients with breast cancer.[42]

Since the first reports in the early 1980s, many studies have confirmed that patients with established breast cancer have measurably lower levels of melatonin.[43,44] While these retrospective trials consistently demonstrate lower melatonin levels in women with breast cancer, it is unclear whether this is due to overutilization or underproduction of the hormone. Clinically, this association may serve to support the application of oral melatonin in patients with existing breast cancer, particularly those with concomitant sleep difficulties.

Oral Supplementation of Melatonin

Much of the evidence supporting the use of melatonin in treating various cancers comes from clinical trials done by Paolo Lissoni of Italy. He has demonstrated a wide array of beneficial effects of high dose melatonin (10–40 mg daily before bed) in patients with advanced cancers of varying origin.[45,46,47,48,49] For a review of these studies, the reader is referred to Lissoni's 2002 article titled Is there a Role for Melatonin in Supportive Care?[50] There have been two trials by his group on the use of high-dose melatonin in women with breast cancer, the details of which are below.

In a pilot study by Lissoni in 1995, melatonin showed benefit in women who had not responded to tamoxifen (TMS) therapy alone.[51] Fourteen women with metastatic breast cancer who had not had a clinical response to TMX were given 20 mg of TMX at noon and 20 mg of melatonin in the evening. A partial response, defined as CT-confirmed reduction of lesions by greater than 50%, was noted in 4 of 14 patients (28.5%) with a median duration of 8 months. Two of these 4 responders had singular lung lesions, one had pleural metastasis, and the fourth had skin metastasis. Stable disease, defined as no objective regression or increase less than 25%, was noted in 8 of 14 patients, 2 of whom had progressive disease on TMX alone. Of note, circulating levels of insulin-like growth factor (IGF-1) decreased in all patients on melatonin, with a significantly greater decrease in those who had a clinical response. Further, 6 of the patients enrolled, and 2 of the 4 responders were estrogen receptor–negative, yet had previously been given tamoxifen due to ineligibility for poly-chemotherapy approaches. This study was done prior to the advent of aromatase inhibitors, which would certainly be applied today to postmenopausal patients who have progressive disease on tamoxifen. Nevertheless, this small trial demonstrates the possible use of a nontoxic agent to enhance the effectiveness of an otherwise ineffective therapy.

The second breast cancer trial by Lissoni's group evaluated high-dose melatonin in women receiving epirubicin chemotherapy weekly, but who had to delay treatment due to the limiting toxicity of thrombocytopenia. Fourteen women with thrombocytopenia were enrolled and given 20 mg per evening of melatonin for 7 days before beginning weekly epirubicin treatments. After 4 cycles, the induction phase of melatonin normalized platelets in 9 of 12 evaluable patients. There was no further platelet decline in these patients throughout the chemotherapy treatment. Tumor regression was achieved in 5 of 12 of the patients and no toxicities were noted. This small trial suggests that melatonin may enhance platelet production and decrease thrombocytopenia in breast cancer patients receiving epirubicin. Larger trials confirming this benefit to platelet-depleting drugs, including epirubicin, are needed in order to make such a conclusion.

It should be noted that Lissoni's work has not been replicated to date, and larger, well-controlled trials are needed to confirm that oral supplementation with high doses of melatonin confers benefit in patients with breast cancer. Also of note is that none of Lissoni's randomized controlled trials were done in a blinded fashion. Nonetheless, a meta-analysis of 10 high-dose melatonin trials done across a variety of cancers reduced the risk of death at 1 year (RR=0.66 CI=0.59–0.73), an impressive trend for an agent with such a low toxicity profile.[52]

Conclusion

Melatonin's role as an oncostatic and cytotoxic agent is well established. In regard to breast cancer, the increased risk of developing breast cancer in women working night/ swing shifts is demonstrated through numerous epidemiological studies. Prospective studies, assessing the incident risk of breast cancer, have corroborated the role of low melatonin as a risk factor through measurement of urinary levels. Further, there may be an inverse association between melatonin levels and tumor size in women with existing breast tumors. From a mechanistic perspective, melatonin's net antiestrogenic effects and anti-inflammatory, immune-modulating, and antimitotic actions continue to lend credence to the potential use of supplemental melatonin as an oncostatic agent in breast cancer. While definitive clinical trials on the effects of supplemental melatonin in breast cancer patients are yet to be done, it has been well demonstrated to be nontoxic at pharmacological doses; this low toxicity profile coupled with the benefits demonstrated in patients with various cancers including breast, make it a candidate for consideration in breast cancer patients, particularly those with late-stage disease.

For more research involving integrative oncology, click here [1].

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