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Urinary Excretion of Melatonin and Association with Breast Cancer: Meta-Analysis and Review of the Literature

Michelle Basler[a,b] Alexander Jetter[a] Daniel Fink[b] Burkhardt Seifert[c]
Gerd A. Kullak-Ublick[a] Andreas Trojan[a,d]

[a]Department of Clinical Pharmacology and Toxicology,
[b]Department of Gynecology, University Hospital Zurich,
[c]Division of Biostatistics, Institute of Social and Preventive Medicine, University of Zurich,
[d]Breast-Center Zürich and Onkozentrum Klinik im Park, Zurich, Switzerland

Keywords

Melatonin · aMT6s · Urinary excretion · Meta-analysis · Breast cancer

Summary

Background: Melatonin is an endocrine hormone secreted by the pineal gland during night hours that provides several biological functions in the circadian rhythm of humans. Due to anti-estrogenic properties, melatonin is considered to exhibit a protective role against the development of breast cancer (BC). Moreover, disruption of melatonin production through environmental influences, such as night work, is assumed to be a risk factor for BC. **Materials and Methods:** We reviewed recent findings concerning biological effects of melatonin on BC and conducted a meta-analysis to evaluate the association between melatonin and BC incidence. In random and fixed effects statistical models, concentrations (tertiles, quartiles) of the primary urinary metabolite of melatonin, 6-sulfatoxymelatonin (aMT6s), were tested for the assumption that women with the highest values would exhibit a lower risk of BC. **Results:** Statistical analysis of data from 5 prospective case-control studies indicates an inverse association between BC risk and the highest levels of urinary aMT6s. This effect seems to be influenced by lag intervals between aMT6s collection and the occurrence of BC, timing and methods of urine sampling, as well as genetic and environmental factors. **Conclusion:** On the basis of the results of our meta-analysis, melatonin is likely to affect BC occurrence in women. However, methodological dissonances may require further studies.

Introduction

Melatonin (N-acetyl-5-methoxytryptamine), an endocrine hormone that is secreted primarily by the pineal gland during night hours, provides several biological functions in the circadian rhythm of humans. In 1978, Cohen et al. [1] proposed the hypothesis that a reduced function of the pineal gland and thus a concomitant decrease in melatonin levels might promote the development of breast cancer (BC) in humans. Later, Tamarkin et al. [2] and Bartsch et al. [3] found an inverse relationship between nocturnal plasma melatonin peak concentrations in women, according to Tamarkin et al. [2] particularly in those with estrogen receptor (ER)-positive BC. Several studies also described lower rates of BC occurrence in blind women, thereby indicating a causative relationship between lower BC rates and an unaffected melatonin secretion [4–6]. Meanwhile, melatonin was demonstrated to modulate the estrogen-signaling pathway in hormone-dependent mammary tumors and to downregulate gonadal estrogen synthesis [7]. Melatonin acts as a selective estrogen receptor modulator (SERM) and prevents estrogen-induced effects by disrupting the binding of the estrogen-ERα-calmodulin complex to the ER-binding element on DNA, thereby inhibiting estrogen-related transcription [8, 9]. Physiological concentrations of melatonin also decrease aromatase expression in MCF-7 human BC cell lines, and promote a synergistic anti-proliferative effect with tamoxifen in anti-tumoral endocrine therapy [10, 11]. Probably unaffected by expression of melatonin MT1 receptors on BC cells, administration of melatonin at physiological concentrations also abrogates extragonadal steroid production via aromatases [12, 13]. Several animal models demonstrated that melatonin administration significantly reduces the occurrence of mammary tumors [10], in relation to which a physiological circadian synchronization seems to be

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PD Dr. med. Andreas Trojan
Breast-Center Zürich and
OnkoZentrum Klinik im Park
Seestrasse 214, 8008 Zürich, Switzerland
andtrojan@yahoo.de

superior to single daily exogenous melatonin application in terms of disease progression [14, 15]. Melatonin was also found to protect animals from adriamycin-induced cardiotoxicity, while co-administration of both substances seems to enhance cytotoxic effects, thereby allowing for dose reductions and attenuation of undesirable side effects [16]. However, a variety of receptor-independent anti-carcinogenic effects of melatonin, such as free radical scavenging via antioxidant properties and modulation of immune functions [17], might exist as well that render the drug potentially useful in the treatment of other diseases.

In 2007, the WHO International Agency for Research on Cancer (IARC) classified 'shift work that involves circadian disruption as probably carcinogenic to humans' [18]. While several studies on mammalian animal models confirmed carcinogenicity of light during the biological night, evidence in humans appears fragmentary [19, 20]. However, an increased risk for BC has been observed among women who worked periods of rotating night shifts [21], although this effect seems to be related to a high quantity of night shifts and the cumulative number of years (> 6 years) a person has worked nights, and in particular depends on whether a night worker is a morning chronotype [22]. In contrast, a recent study [23] associated an increasing incidence of BC with small numbers of night shifts per week, indicating a potential relationship to more frequent disruptions of the circadian rhythm with deranged adaption to changes between day and night schedules [24]. In young women, a night shift-associated BC risk might even exist before the first full-term pregnancy since the mammary gland is not fully differentiated until first childbirth and lactation [25].

Whether relative concentrations or the absolute decrease of melatonin levels with increasing years of sleep disturbance as well as sleep duration might affect the risk of BC remains inconclusive [26–31], since many studies do not provide quantitative urinary or serum melatonin concentrations. Independent of melatonin dynamics, interpretation of studies might be complicated by the expression of circadian clock genes, such as cell cycle regulators p53 and c-myc, which directly affect apoptosis and proliferation, as well as potential differences in culture, diet, immune reactivity, and genetic variations [32, 33]. Here we provide an overview of the recent findings concerning the biology and impact of melatonin in relation to BC occurrence. A meta-analysis was conducted to quantitatively assess a possible association between melatonin and BC incidence based on studies of urinary melatonin concentrations.

Materials and Methods

Melatonin is metabolized in the liver and exhibits a short half-life of about 40 min [34]. The main degradation pathway consists of 6-hydroxylation followed by conjugation for renal elimination. The concentration of the primary urinary metabolite of melatonin, 6-sulfatoxymelatonin (aMT6s) reflects the amount of nocturnal plasma melatonin in the body [35]. Quantitative assessment of aMT6s in urine is deemed a practical method to determine the circadian phase since it represents the amount of endogenous melatonin during collection time and is thus considered a reliable approximation of melatonin production in an individual, even if urine is stored over prolonged periods of time [36]. Although small phase changes could be monitored by frequent sampling of blood or saliva [37], simple morning urine aMT6s measurement does not interfere with the participant's sleeping pattern. Despite a large variability in melatonin amplitudes, their range remains relatively stable within the same person [38], justifying singular melatonin measurements as is conducted in most recent studies. For our meta-analysis based on urinary melatonin concentrations, 2 sampling techniques were applied: first morning urine and 24-h urine collection. Studies that focused on aspects such as duration of sleep and tumor size were excluded in the numerical analysis. The data source for the meta-analysis was provided by the electronic database PubMed, and our review comprises only English articles from 1989 to 2013. Search terms included melatonin, breast cancer, and urinary sulfatoxymelatonin (aMT6s).

Statistical Considerations and Results

A total of 24 search results were found of which 10 studies measured the urinary aMT6s concentration as a surrogate of circulating melatonin levels: Skene et al. (1990) [39], Bartsch et al. (1997) [40], Travis et al. (2004) [41], Schernhammer et al. (2005) [42], Wu et al. (2008) [31], Schernhammer et al. (2008) [43], Schernhammer et al. (2009) [44], Schernhammer et al. (2010) [45], Davis et al. (2012) [46], and Wu et al. (2013) [47]. Of these 10 studies, 5 displayed their data in a similar manner and met the criteria of this meta-analysis (table 1).

Table 1. Overview of the studies used for the present meta-analysis

First author [ref.]	Year	Data	Urine sample	Menopausal status	Country or cohort and time period of enrollment
Travis [41]	2004	tertiles	24-h urine	pre- and postmenopausal	Island of Guernsey (British crown dependency), Guernsey III Study; 1977–1985
Schernhammer [42]	2005	quartiles	first morning urine	primarily premenopausal	USA, Nurses' Health Study II (NHSII) cohort (1989); 1996–1999 (urine collection)
Schernhammer [43]	2008	quartiles	12-h overnight urine	postmenopausal	Italy, Hormones and Diet in the Etiology of Breast Cancer Risk cohort (ORDET cohort, postmenopausal); 1987–1992
Schernhammer [44]	2009	quartiles	first morning urine	postmenopausal	USA, Nurses' Health Study (NHS) cohort (1976); 2000–2002 (urine collection)
Schernhammer [45]	2010	quartiles	12-h overnight urine	premenopausal	Italy, Hormones and Diet in the Etiology of Breast Cancer Risk cohort (ORDET cohort, premenopausal); 1987–1992

Table 2. Data used for urinary melatonin meta-analysis (Event 1 = cases in the highest quartile, Event 2 = cases in the lowest quartile, n = total of respective quartile; case subjects defined as women who developed breast cancer after their enrollment in the study cohort; matched control subjects were randomly chosen, alive, and free of cancer at the time of diagnosis of the index case subject)

First author [ref.]	Year	Event 1	n 1	Event 2	n 2	Comment
Travis [41]	2004	46	163	39	158	tertiles, 24-h urine, pre- and postmenopausal
Schernhammer [42]	2005	23	96	50	123	first morning urine, primarily premenopausal
Schernhammer [43]	2008	40	218	56	233	12-h overnight urine, postmenopausal
Schernhammer [44]	2009	75	210	107	243	first spot morning urine, postmenopausal
Schernhammer [45]	2010	30	113	28	93	12-h overnight urine, premenopausal

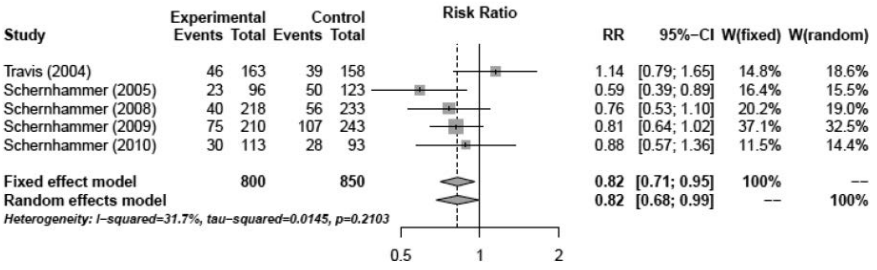

Fig. 1. Forest plot illustrating the combined estimated risk ratio for breast cancer from the meta-analysis.

To evaluate a potential publication bias, a funnel plot was constructed. The linear regression test of funnel plot asymmetry was performed and suggests that the data is distributed evenly and there is no indication of a selection bias (p = 0.94). Although the number of studies seems too small to exclude selection bias, the p value and funnel plot (figures not shown) do not indicate evidence thereof.

Conclusions drawn from these 5 evaluable studies can be summarized in brief as follows: Travis et al. [41] found no significant difference in urinary melatonin concentrations in BC patients and controls (Guernsey III study). In 2005 (NHSII) and 2008 (ORDET cohort), Schernhammer et al. [42, 43] reported that increased melatonin levels were statistically significantly associated with lower risk of invasive BC in pre- and postmenopausal women. This inverse association was again confirmed by Schernhammer et al. in 2009 (NHS) [44], while in 2010, Schernhammer et al. also reported a positive association between aMT6s concentration and BC risk in premenopausal women (ORDET cohort) [45]. In particular, when cases were excluded which exhibited a lag time below 2 years between urine collection and BC diagnosis, melatonin had a protective effect on premenopausal BC, i.e. an inverse association could be observed [45]. Since most samples from the recent study by Wu et al. [47] were not derived from first morning voids but from randomly timed spot urine, unfortunately, this study was deemed not suitable for our meta-analysis.

Upon statistical considerations concerning these studies, melatonin concentrations were classified into quartiles or tertiles according to the one-time overnight urinary aMT6s concentration (table 2). Since our assumption was that women in the highest quartile (exhibiting the highest melatonin values) would experience a lower risk of developing BC compared to women in the lowest quartile (i.e. exhibiting a risk ratio smaller than 1), the relative BC risks of the highest and lowest quartile (tertile respectively) were determined, compared, and used to calculate the corresponding odds ratios.

An important factor, which was taken into account, is the lag time between urine sample collection and diagnosis of BC. Since the presence of a preclinical tumor might have influenced the basic melatonin level, 2 studies [41, 45] excluded individuals in which cancer diagnosis occurred within less than 2 years of urine collection (data not shown). However, Travis et al. [41] reported unchanged results when an adjustment for a 2-year lag time was performed. In the 2008 study by Schernhammer et al. [43], the mean time between urine collection and diagnosis was 80 months (± 50 months standard deviation (SD)), while in Schernhammer et al. 2009 [44] this time interval was only 30 months (± 18 months SD).

Using the data with a 2-year lag time (where specified), led to the following considerations: To decide whether to use a fixed or random effects model in this meta-analysis, a test of heterogeneity (Cochran's Q) was performed and produced a p value of 0.21. In meta-analyses with a fixed effect, it is assumed that the same effect underlies each study and that the observed variability occurs only due to sample variability. However, with a p value (obtained from the test of homogeneity) higher than 0.05, the null hypothesis cannot be rejected, i.e. this p value indicates no evidence against homogeneity. Consequently, the fixed effects model could be applied. As mentioned above, the sample size is considered small, and hence the Cochran's Q test has low power. In addition to sample variability, however, analysis may also be performed on the assumption that the underlying effect between the studies varies randomly [48]. Using the random and fixed effects models, a relative risk of 0.82 was obtained. Statistical significance is attained in the random as well as in the fixed effects model: 95% confidence interval (0.68; 0.99), p value 0.04 for the random effects model, and 95% confidence interval (0.71; 0.95), p value 0.01 for the fixed effects model. Although not

all included studies suggest an effect in the same direction, the confidence intervals are overlapping in all of them. The combined estimated risk ratio is smaller than 1 and hence implies that a reduced BC risk prevails in women with higher melatonin levels (fig. 1).

Discussion

We identified 5 prospective case-control studies that met the inclusion criteria and were finally used for statistical calculations. Although Schernhammer is co-author in 4 of the 5 studies, their data sets can be considered different from each other (table 1, funnel plot). The analysis also revealed the importance of a defined lag time between urine collection and BC diagnosis in order to appropriately assess combined risk evaluations for BC upon urinary melatonin excretion [45]. In this regard, the ORDET cohort in premenopausal women found highly variable odds ratios, while a significant BC risk reduction of 34% in the highest category of aMT6s levels (combined estimate 0.66) was obtained only when data with a lag time of > 2 years were analyzed [41, 45, 47]. In contrast, adjustment for a 2-year lag time presented unchanged results in the study by Travis et al. [41]. However, the studies by Travis et al. [41] and Wu et al. [47] potentially failed to demonstrate a protective effect of melatonin due to the variable timing of sample collection; both studies were based on measurement of non-timed urine collections (24-h and randomly timed spot urine). Considering the importance of lag-time, our meta-analysis apparently displays a trend towards a protective effect of melatonin on BC occurrence, although only marginally significant (fig. 1).

Biological effects of melatonin might also be compromised by mutations in the melatonin receptors (MTNR1a and MTNR1b) as well as alterations of the enzyme arylalkylamine N-acetyltransferase (AA-NAT) which catalyzes a key step of melatonin synthesis in the pineal gland, and gets rapidly inactivated when exposed to light at night [49, 50]. Whilst a genome-wide association study from the Shanghai Breast Cancer Study discovered single nucleotide polymorphisms concerning the melatonin receptor expression, an association with variable susceptibility to BC may correspond to different phenotypes within a population of the same racial origin [51]. Consequently, results and dynamics of melatonin concentration from Chinese cohorts [47] need to be interpreted with respect to epigenetic modifications, menopausal status, and dietary as well as environmental variables such as sleep deprivation and compromised immune function [52–54].

While most studies and our meta-analysis evaluated melatonin levels before BC occurrence, assessments of melatonin levels in patients with manifest cancer indicate that the tumors themselves might affect melatonin secretion upon systemic endocrine reactivity towards cancer. Higher melatonin levels seemed prevalent in untreated BC patients and those with a favorable prognosis, i.e. ER-positive and node-negative tumors [55–57], although this trend was no longer seen in older and postmenopausal women in whom circulating aMT6s levels seem to decline [39]. In contrast, low aMT6s excretion was reported with increasing tumor size, and the melatonin amplitude normalized again after tumor removal [3, 40].

So far, available studies provide only little information on how to determine which collection time would optimally reflect biological effects of melatonin. Although urinary aMT6s is deemed an appropriate metabolite associated with non-invasive collection procedures, it only represents a surrogate for endogenous periodical melatonin secretion. Since studies measured aMT6s concentrations e.g. over 24 h, 12 h, or in the first morning urine as well as randomly timed spot urine, that information cannot reliably be combined for calculations. Moreover, 24-h urinary melatonin assessment may reflect the nocturnal increase in melatonin concentrations, but does not consider peak and minimum values. Wu et al. [47] therefore suggested that randomly timed spot urine samples are not suitable as a surrogate for melatonin concentrations in humans and even provide an inaccurate comparison for women in the same cohort. Since it remains unclear to what extent a preclinical breast tumor might influence melatonin concentrations, supplemental information on lag times seems mandatory [39, 41, 47].

Thus, further investigations need to evaluate methods of melatonin assessment in order to facilitate the comparison of different studies and ethnic groups with a focus on longitudinal analysis and dynamics of melatonin secretion in individuals before tumor discovery and under treatment. Finally, clinical trials designed to exogenously administer melatonin may be able to confirm effects of melatonin on BC incidence and provide information on dosage and long-term safety. For this, appropriate (range between the 75th and 100th percentile) levels of urinary aMT6s could be achieved e.g. by administration of synthetic melatonergic agonists with extended half-lives [34, 58]. Alternatively, filter glasses and light systems that block blue spectra and short-term exposure to bright artificial light should protect shift workers from an unintended melatonin decline [20, 59–61]. In the light of the present trend towards a 24-h society, the current data on the effects of melatonin may warrant further investigations with a focus also on cancer prevention in men [62].

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Disclosure Statement

All authors and coauthors indicated no potential conflicts of interest.

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