Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Circadian rhythm of melatonin in postmenopausal asthmatic women with hormone replacement therapy

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Submitted: *September 30, 2001*
Accepted: *February 28, 2002*

Key words: **melatonin; circadian rhythm; hormone replacement therapy; asthma bronchiale**

*Neuroendocrinology Letters 2002; **23**:243–248 pii: NEL230302A07*

Abstract

OBJECTIVE: The aim of the study was to check if in postmenopausal women such a disease as asthma and use of hormone replacement therapy (HRT) influence daily melatonin (MEL) secretion.

MATERIAL AND METHODS: Studies were performed in 55 asthmatics (treated and not treated with glucocorticosteroids) and 20 healthy postmenopausal women (aged 48–60) before HRT and after 6 months of transdermal 17β-estradiol and medroxyprogesterone acetate treatment (cyclical method). During the circadian study blood samples for the measurement of MEL were collected every 3 hours during the day. MEL concentrations were assessed with the use of RIA methods. Statistical analysis of the circadian rhythm was performed with the use of cosinor test according to Halberg et al.

RESULTS: Existence of daily rhythm of MEL secretion was shown in all studied groups, before as well as after HRT. A significant decrease of mesor and amplitude's rhythm was observed in the group of women with asthma treated with glucocorticosteroids (GC) before and after HRT use in relation to women not treated with GC and the control group. Secretion of MEL in these patients was lower at nocturnal hours. A significant decrease of mean daily MEL secretion in relation to values before HRT use was shown in all groups.

CONCLUSIONS: Asthmatic postmenopausal women treated with GC show lowered circadian secretion of melatonin as a consequence of lowering its secretion at nocturnal hours. Hormonal replacement therapy causes a decrease of daily melatonin secretion in healthy as well as asthmatic women, not disturbing circadian rhythm of this hormone's secretion.

ABBREVIATIONS

A	amplitude
E_2	estradiol
GC	glucocorticosteroids
GnRH	gonadotropin-releasing hormone
HPA	hypothalamo-pituitary-adrenal
HRT	hormone replacement therapy
M	mesor
MEL	melatonin
LH	luteinizing hormone
p	probability
RIA	radioimmunological methods
φ	acrophase
ω	angular frequency

Introduction

The role of melatonin (MEL) in both physiological and pathological states in humans remains unclear.

The best-documented role of MEL is regulation of the circadian rhythm of biological processes. It may have a variety of other functions: free radical scavenger, transitional metal chelation, immuno-stimulation, neuroprotection, antitumor and osteoblast promotion [1]. MEL levels fall markedly with ageing, and may contribute to the incidence or severity of some age-associated neurodegenerative diseases [2].

Several lines of evidence indicate that ageing and gonadal steroids influence biological responses to MEL. Gonadal steroids modulate the number of MEL receptors in animals and influence LH, cortisol and body temperature responses to exogenous MEL in humans [3].

A direct modulatory effect of the gonadal hormones on pineal MEL synthesis is well established in animal studies. In the case of humans abnormal MEL release associated with disorders of the reproductive system can only be argued in the presence of compelling evidence suggesting a relationship between MEL and hypothalamo-pituitary-gonadal axis. The demonstration of MEL receptors on different gonadal cells from various species as well as seasonal variation in gonadotropins and gonadal steroids receptors in the human pineal gland and negative significant correlation between the peak serum MEL and serum 17β-estradiol in perimenopausal women further strengthen the relationship between MEL and reproductive hormones, although it is not known whether these receptors and their ligands are crucial to pineal MEL secretion [4]. Recent studies indicate also other connections between MEL and estrogens significant for organism. Estrogens exert pro-oxidative effects and have been shown to damage DNA, potentially leading to cancer. MEL is a well-known antioxidant and oncostatic agent. Studies on animals confirmed the dual actions of estrogens relative to oxidative damage, i.e., estrogen increases oxidative destruction of DNA while reducing lipid peroxidation. MEL had antioxidative actions in reducing oxidative damage to both DNA and to membrane lipids. MEL completely prevented the damaging action of E2 on DNA and synergized with the steroid to reduce lipid peroxidation [5].

It has been reported that several biological responses to MEL can be influenced by the estrogenic and possibly progestogenic environment, and are sometimes inconsistent in elderly postmenopausal women [6].

There is still a considerable amount of uncertainly related to the application of hormone replacement therapy (HRT) regarding its influence on hormonal changes occurring in the organism of healthy women as well as in the course of many diseases in the postmenopausal period. There are also few reports concerning the influence of HRT on MEL secretion in this period.

Cagnacci et al. [3] indicated the circulatory response to MEL is conserved in postmenopausal women with, but not without, HRT. Maintenance of the cardiovascular response to MEL may be implicated in the reduced cardiovascular risk of postmenopausal women with HRT. MEL influences vascular reactivity and reduces blood pressure and norepinephrine levels [7]. The same authors [3] also observed that MEL does not modulate adrenergic activity in postmenopausal women without HRT. Estradiol replacement restores the capability of MEL to modulate adrenergic activity, particularly the norepinephrine response to stimuli.

Because the postmenopausal period condenses age- and hypoestrogenism-related biological modifications, we investigated whether in postmenopausal women disease such as asthma and use of HRT influence daily MEL secretion.

Material and methods

Studies were performed in 55 postmenopausal asthmatic women aged from 48 to 66 [mean 52.93±3.16 years).

Women: over 60 years old, with postmenopausal period lasting more than 5 years, smokers, with neoplastic, kidneys, liver (among others cholecystolithiasis), heart and vessels (among others fixed hypertension, after past or recent thrombotic episodes of arterial and venous vessels), metabolic (among others with diabetes), haematological, endocrinologic (not being asthma and glucocorticotherapy complications) and gynaecological (among others bleeding from genital ways of unknown origin and with endometriosis) diseases were excluded from the studies.

Asthmatic women were divided depending on the severity of asthma and used the therapy according to Guidelines for the diagnosis and management of asthma (National Heart, Lung and Blood Institute 1997) [8], into the following groups:

- First group (I) consisted of 32 women with moderate asthma, using glucocorticosteroids (GC) in inhalative form (Budesonide in medium doses 600–1600 μg/day). Oral GC (Polcortolon 8–12 mg/24h) were administrated during asthma exacerbations, but not

longer than 7 days a month. These women had not chronically taken systemic GC for at least 2 years;
- Second group (II) consisted of 23 women with sporadic and mild asthma not treated with GC;
- Control group (III) consisted of 20 healthy postmenopausal women aged from 49 to 58, not treated with other drugs except HRT.

The clinical state of all patients assessed with clinical examination on the day of blood uptake for hormonal assessment was good. Any hormonal drug containing estrogens or gestagens was used before studies for at least 6 months. All women before and during HRT use were subjected to detailed examination conducted by an internist and gynaecologist. Contraindications to the use of HRT were not noticed in any of the women. No other steroid drugs than mentioned above or antidepressive drugs were taken during the studies. Women participating in studies signed an agreement for them.

The local Ethic Committee's agreement was obtained for the study.

Studies of daily hormone concentrations were done before HRT application and after six 28-day cycles of therapy with 17β-estradiol and medroksyprogesterone acetate using cyclic method. Transdermal 17β-estradiol (Estroderm TTS-firm Novartis or Systen-firm Cilag) was used in daily dose in the form of plasters stuck twice a week from the 1st to the 21st day of the cycle. Medroksyprogesterone acetate (Provera) was administrated orally in a dose of 10 mg/day from the 11th to the 22nd day of the cycle.

Circadian MEL concentration assessment after HRT was carried out between the 18th and the 20th day of the sixth cycle. During the studies women were hospitalized before as well as after HRT use. Vein blood for hormone assessments was collected every 3 hours during the day.

MEL concentrations were measured with the use of commercially available RIA kits produced by DRG Instruments GmbH (USA). Sensitivity of the assay, intra- and interassay coefficients of variations were of 1pg/sample, 8.6% and 9.2%, respectively.

Statistical analysis

Statistical calculations were performed with the use of a personal computer (CSS Statistica program). Wilcoxon matched pair test was used for comparison of the mean variable of studied values for two trials. The results were statistically analysed with the use of variance analysis for Kruskal-Wallis nonparametric tests. After rejecting the variance uniformity hypothesis, further analysis of statistical significance was conducted using "U" Mann-Whitney test.

The statistical analysis of the circadian rhythm was performed with the use of a cosinor test according to Halberg et al. [9]. Cosinor analysis was carried out for a fixed average time group value by fitting the main cosinor function $f(T)=M+A\cos(t\omega+\varphi)$, where $f(T)$ is the average hormone concentration at the given time point; M is the mesor, arithmetic average of actual values describing oscillations within the cycle; A is the amplitude, difference between the maximum (or minimum) value and the sinusoidal average; φ is the acrophase, angle ($360°=24h$) corresponding to the maximum value of a given hormone concentration within 24 hours; ω is angular frequency. The appearance of a rhythm was deducted following rejection of zero amplitude hypotheses.

Results

Cosinor analysis of MEL secretion during the day showed existence of daily rhythm in the three studied groups, before as well as after HRT (table 1).

In group I of women treated with GC, before HRT use, a significant decrease of mesor and amplitude's rhythm and slight displacement of acrophase (0 h 51') in relation to control group was observed (tab.1A). A significant decrease of mean daily MEL secretion in group I in relation to group II and the control group before HRT is illustrated in Figure 1.

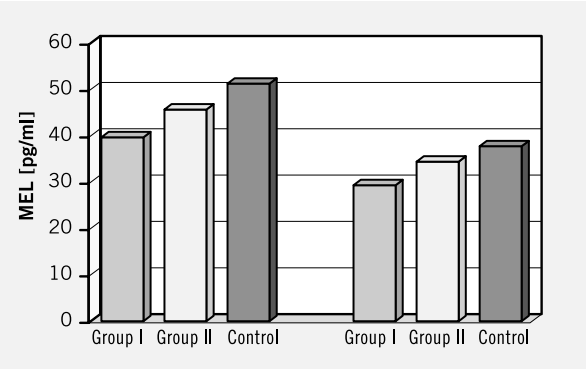

Fig. 1. Comparison of mean daily values of melatonin (MEL [pg/ml]) concentrations in serum of asthmatic patients, treated with glucocorticosteroids (group I), not treated with glucocorticosteroids (group II) and in the control group (III), before and after hormonal replacement therapy use (HRT).

Table 1. Chronobiological parameters of circadian rhythm of melatonin secretion in asthmatic patients treated with GC (group I), not treated with GC (group II) and in the control group (III)

A) before HRT use

HORMONE	GROUPS	MESOR [pg/ml]	AMPLITUDE [pg/ml]	ACROPHASE [h, min]	COSINE
Melatonin	I	39,68*♦	21,11*♦	0 h 51′	p<0,001
	II	45,66	41,54	1 h 33′	p<0,001
	III	51,26	41,30	2 h 01′	p<0,001

B) after HRT use

HORMONE	GROUPS	MESOR [PG/ML]	AMPLITUDE [PG/ML]	AKROPHASE [H, MIN]	COSINE
Melatonin	I	29,91*♦	21,90 *♦	0 h 59′	p<0,001
	II	34,78	31,77	1 h 40′	p<0,001
	III	37,75	31,00	1 h 54′	p<0,001

* p≤ 0,05 group I vs. group II
♦ p≤ 0,05 group I vs. group III

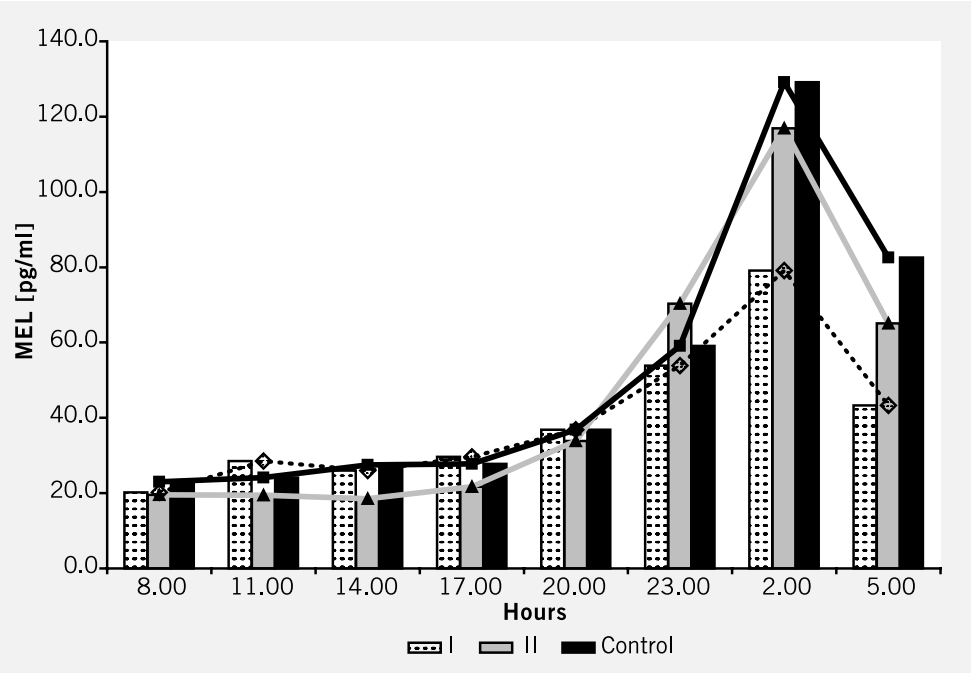

Fig. 2. Circadian profile of MEL concentrations at particular hours of the day in asthmatic women and in the control group before HRT use.

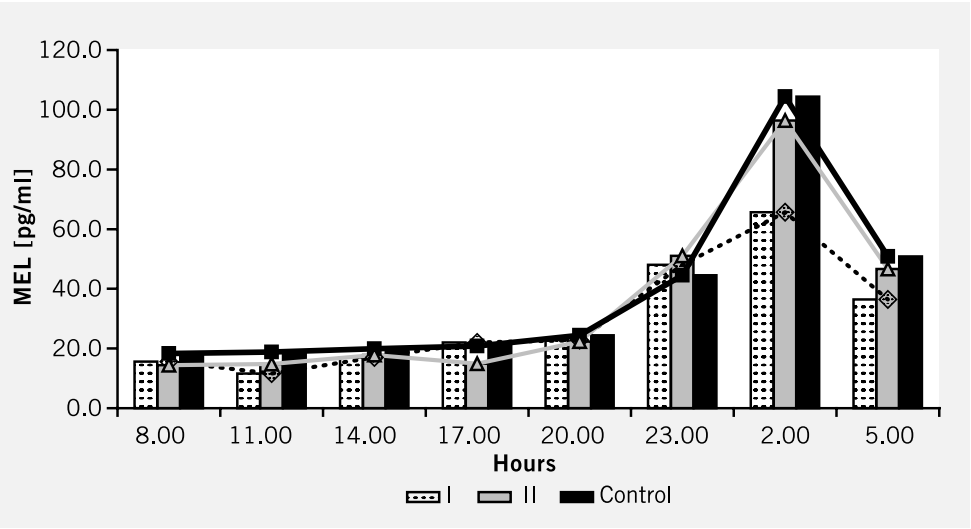

Fig. 3. Circadian profile of mean daily MEL concentrations at particular hours of the day in asthmatic women and the control group after HRT use.

Mean daily values of MEL concentrations in serum in the group of patients not treated with GC did not significantly differ in relation to values observed in the control group before as well as after HRT (figure 1).

Circadian oscillations of mean MEL concentrations at respective hours of the day in asthmatic women and the control group before and after HRT use are presented in Figures 2 and 3.

In the group of women treated with GC before HRT use a significant decrease of MEL secretion at 2 am and 5 am in relation to the group of patients not treated with GC and the control group was shown, however in the group of patients not treated with GC statistically lower in relation to control group values of MEL concentrations were observed only at 2 am (fig.2).

After HRT use, similarly as before therapy use, mesor values were lowered in the group of patients using GC in relation to group II and control group. Significant lowering of amplitude's rhythm in group I in relation to group III was observed, however acrophase of a rhythm was displaced for more than an hour (table 1B).

In group I, after HRT use, a significant decrease of MEL concentrations in relation to group of patients not treated with GC and the control group was observed only at 2 am, yet MEL secretion at 5 am was lowered only in relation to the control group (fig.3).

Significant lowering of mean daily MEL secretion in relation to values before HRT use was shown in all the studied groups (fig.1).

Discussion

Similarly as in our earlier studies [10] we have shown the existence of daily rhythm of MEL secretion in asthmatic women.

Postmenopausal asthmatic women treated with glucocorticosteroids (GC) had lower mean daily MEL secretion in relation to patients not treated with GC and healthy women. It was mainly a result of lowering of MEL secretion in nocturnal hours. These observations are compliant with earlier studies, in which we have shown in women before menopause receiving long-term corticotherapy, that the levels of MEL were significantly lowered as compared to patients not treated with GC and to the control group [10]. These results are compliant with reports obtained by some researchers, which support the hypothesis of an inhibitory influence of exogenous glucocorticoids on the secretion of pineal gland hormones [11,12]. Demisch et al. [13] showed that the administration of synthetic GC significantly lowers nighttime production of MEL also in healthy subjects.

Ageing and hypoestrogenism are believed to impair the regulation of the hypothalamo-pituitary-adrenal (HPA) axis and may participate in the determination of this altered response [14]. Cagnacci et al. [14] revealed that in postmenopausal women, reversal of hypoestrogenism, resulting from supplemental estrogens, might improve the regulation of the HPA axis. It was also shown, that gender and aging may modulate the melatonin effect on cortisol. In aged postmenopausal women the cortisol levels are enhanced at selected circadian times and are stimulated by melatonin [15].

Studies on animals testify for reciprocal connections between HPA and MEL axis. MEL administration was associated with diminished overall corticosterone secretion and increased sensitivity to glucocorticoid feedback. These findings indicate that chronic MEL treatment may protect several regulatory components of the HPA axis from glucocorticoid-induced deterioration [16].

In asthmatic women a tendency to decrease not only MEL, but also other hormones secretion, among others estrogens, cortisol or dehydroepiandrosterone sulphate, was demonstrated [17,18].

In postmenopausal women the age-related decline of MEL levels is observed, which may be associated with a reduced biological response to the hormone. Levels of MEL are reduced in the course of various diseases, for example in individuals with coronary artery disease and also in elderly subjects, in which the incidence of cardiovascular disease increases [6].

It was proved in other studies that a steep, age-related decline in nocturnal MEL secretion among postmenopausal women was found for up to 15 years postmenopause, followed by an extremely gradual decline thereafter. A low estrogen state, induced by oophorectomy of premenopausal women with uterine leiomyoma led to an increase in nocturnal melatonin secretion. These findings suggest that transient elevated nocturnal melatonin secretion during menopause may be related to the existence of a low estrogen environment. The age-related decrease in melatonin secretion observed in other conditions is most likely attributable to other age-related factors [19].

We have shown in our studies that HRT does not cause disturbances in daily rhythm of MEL secretion, however there is a significantly lowers mean daily secretion of MEL in relation to values before HRT use in all the studied groups.

The results of our studies are compliant with the results of Okatani et al. [19], according to whom daily oral administration of conjugated estrogen (0.625 mg) to postmenopausal women suppressed nocturnal MEL secretion.

Similar observations were made by Okatani and Sagara [20] in patients with secondary amenorrhea, who observed that nocturnal MEL concentrations were significantly higher than in women with normal menstrual cycles. There were significant negative correlations between cumulative melatonin levels (between 8 p.m. and 8 a.m.) and serum 17β-estradiol and between peak serum MEL values and serum E_2 concentrations in women with secondary amenorrhea. Intravenous administration of a conjugated estrogen (Premarin 20 mg) significantly suppressed nocturnal MEL secretion. These findings suggest that elevated nocturnal MEL secretion may be related to low estrogen levels and estrogens administration diminishes its secretion.

In favour of E_2 modulating MEL secretion are the finding of decreased MEL after E_2-replacement in GnRH deficient women and the elevation of MEL in E_2 deficient women with endometriosis receiving GnRH analogue therapy [21]. The efficacy of E_2 in modulating MEL secretion was not confirmed in other studies [22]. Bartsh et al. [23] investigated also the influence of two routes of estradiol administration on pineal MEL production in postmenopausal women. Both transdermal and oral estradiol treatment led to an increase as well as decrease of MEL production in different patients. These investigators [23] observed that the reason why individuals respond either in a stimulatory or inhibitory manner is unknown and requires evaluation in further more extensive studies.

To sum up, it may be stated that decreased MEL secretion in asthmatic women can have therapeutic implications. It was shown that MEL might be helpful in many diseases, which accompany postmenopausal asthmatic women, among others in cardiovascular disease, probably because of its free radial scavenger activity [2]. MEL may also decrease cholesterol levels and improve fatty changes in the liver. Besides antioxidant properties, the pineal hormone MEL influences several endocrine and biological functions

[24,25,26,27,28,29]. It was also shown that in postmenopausal women, administration of 1 mg of MEL reduces glucose tolerance and insulin sensitivity [23].

Conclusions

1. Postmenopausal asthmatic women, treated with glucocorticosteroids, show decreased mean daily melatonin secretion as a consequence of lowering its secretion in nocturnal hours.
2. Hormone replacement therapy causes, in healthy as well as in asthmatic women, decrease of daily melatonin secretion, not disturbing daily rhythm of this hormone secretion.

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