Effects of Melatonin in Perimenopausal and Menopausal Women

Our Personal Experience

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ABSTRACT: The purpose of this clinical trial on possible effects of nocturnal MEL administration in perimenopausal women was to find if MEL by itself modifies levels of hormones and produces changes of any kind, independently of age (42–62 years of age) and the stage of the menstrual cycle. It is accepted that a close link exists between the pineal gland, MEL, and human reproduction and that a relationship exists between adenohypophyseal and steroid hormones and MEL during the ovarian cycle, perimenopause, and menopause. Subjects took a daily dose of 3 mg synthetic melatonin or a placebo for 6 months. Levels of melatonin were determined from five daily saliva samples taken at fixed times. Hormone levels were determined from blood samples three times over the 6-month period. Our results indicate that a cause-effect relationship between the decline of nocturnal levels of MEL and onset of menopause may exist. The follow up controls show that MEL abrogates hormonal, menopause-related neurovegetative disturbances and restores menstrual cyclicity and fertility in perimenopausal or menopausal women. At present we assert that the six-month treatment with MEL produced a remarkable and highly significant improvement of thyroid function, positive changes of gonadotropins towards more juvenile levels, and abrogation of menopause-related depression.

KEYWORDS: melatonin; menopause; aging

A progressive reduction of nocturnal serum melatonin (MEL) concentration is observed in aging healthy humans.[1] It has been demonstrated that the pineal gland is responsible for the development and maintenance of neuroendocrine and sexual functions. In fact, the identification of the key role of the pineal gland in the control of reproductive biology emerged from the observation of precocious puberty in children carrying pineal tumors.[2] In nonhuman mammals, such as sheep, seasonal fer-

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tility and reproduction during young, reproductive age, are strictly dependent on night levels of MEL, which vary according to the seasons (temperature and photoperiod). In this case MEL exerts an inhibitory action on gonadotropins and thyroid hormones.[3–5] The effect of MEL seems to be related, at least to a large extent, to regulation of high-affinity MEL receptors in the neurohypophysis and on modulation of leutotropic hormone-releasing hormone (LH-RH) receptor density in the hippocampus and in the brain in general (anterior hypothalamus, suprachiasmatic nuclei, preoptic area).[6–8] MEL administration at high, pharmacological doses, inhibits gonadal function in male hamsters and this effect is especially evident when MEL is given at daytime rather than with nocturnal periodicity.[9,10] MEL at extremely high doses and for periods of years has also been given to thousands of young women in the attempt to develop a new kind of contraceptive pill without early or late side effects.[11] Also, MEL thwarts the growth of hormone-sensitive tumors, such as certain breast cancers, and can increase the density of hormone receptors on cancer cells.[12]

In the course of aging, age-relate illnesses and disabilities are clearly linked to a progressive decline of reproductive-sexual functions in men (andropause) and women (menopause). The progressive decline of this basic function leads to severe side-effects psychosomatic in nature and to a chain of negative events that stem from the close relationship between neuroendocrine and immune capacity and functions. In fact, it has been demonstrated that LH-RH is a powerful immuno-regulating and immuno-enhancing hormone both in ontogeny and in adult life.[13]

Findings from our laboratory have demonstrated that administration of MEL in the night hours and transplantation of pineal glands from young into older mice and rats maintain juvenile thyroid function and significantly delay their aging and/or prolongs their life.[14,15] A clear-cut activity of MEL and the grafting of young-to-old pineal glands is the maintenance of gonadal and sexual functions. A most remarkable maintenance of juvenile levels of LH-RH receptors both in the brain (hippocampus) and in the gonads is achieved by transplantation of the pineal gland from a young donor rat into older recipients.[16] This effect becomes more pronounced in the course of months after grafting, which demonstrates that, at least in rats, there is more than just a simple return of sexual functions to more juvenile levels.[16] The general conclusion derived from others' and our own work is that circadian, nocturnal administration of MEL may postpone endocrine aging and maintain or reconstitute more juvenile sexual functions at a time of life (e.g., between 40 and 60 years of age in women) when changes of ovarian cyclicity become evident (premenopausal, perimenopausal, and menopausal age). Along with the complete absence of noxious side-effects in MEL treatment, there are many somatic and psychological benefits for women of all ages. Many women, just before and after menopause, undergo a hormone replacement therapy (HRT) to correct levels of ovarian steroids, such as estrogens and progesterone, to premenopause values. HRT also protects against the psychosomatic side-effects of menopause and against heart disease and osteoporosis. Even if MEL could not, by itself, prevent completely menopause and maintain ovarian cyclicity, it may enhance and improve the efficiency of HRT and thus add more years of treatment until there is an opportunity for prevention, delay, or mitigation of menopause and its related psychosomatic problems. An early intervention with night administration of MEL before the initiation of ovarian dysfunction in relatively young women may indicate to us whether or not MEL can modify the onset or the course of menopause in women.

PATIENTS AND METHODS

Patients and Treatment

A formal and fully documented application for the conduction of the clinical trial was delivered to the Ministry of Public Health, Rome, Italy. All women joining the clinical trial were asked to sign an informed consent. They were given detailed information on the character, duration, and aims of the research. Women were carefully selected who had no relevant pathologies, no history of use of drugs, hormones, or herbal preparations, were living a normal lifestyle with typical Mediterranean diet rich of carbohydrates and fresh vegetables, and had the normal sleep habits of the population of the region south of Rome. All women did not smoke and did not abuse alcohol.

All women completed a questionnaire that included questions about life habits, physiological data, data concerning previous pathologies, treatment-related side-effects, treatment-induced alterations, effects on perimenopausal symptoms (neurovegetative, sleep, psychological) were recorded at time 0, and at 3, 6, 12, 24 months of MEL or placebo treatment. The questions concerned duration and character of their menstrual cycle and/or psychosomatic, symptoms associated with perimenopause and menopause (such as irritability, morning mood, depression, insomnia, night sweats, headache, stypsis, amnesia, hot flushes, palpitations, and body weight increase).

The recruited premenopausal (45 women), perimenopausal (56 women), and postmenopausal (38 women) women age ranged from 42 to 62 years of age. They were divided in two age groups (42–49 and 50–62) of similar age and peri- or postmenopausal symptoms. In fact, in most countries and latitudes perimenopausal symptoms and onset of menopause do not depend precisely on the age but rather on individual, environmental, and genetical variability. The two groups of women were given plastic bottles with 60 MEL capsules of 3 mg (MEL, synthetic, 100% purity) or placebo at the beginning of each two-month period. The bottles with MEL or placebo were certified as a GMP (Good Manufacturing Practice) product and were a gift from Eurochem Ltd, Munich, Germany. They were only marked with a code number. The opaque white capsules of MEL and placebo were identical. It was unknown to the women and to the doctor which of the two groups received MEL or placebo. In fact the key of the code number (MEL or placebo) was unknown to the patients and to the doctor and was opened only at the end of the first six-month trial.

All women were asked to take the capsules at bedtime, between 10 and 11 p.m. For obvious ethical reasons, administration of placebo was restricted to the initial six months of this ongoing clinical study.

At the end of the six-month trial, final results were collected from a total of 139 women, with small number variations in the group studied depending on minor failure of some determinations of MEL in saliva, T3, T4, LH, and follicle-stimulating hormone (FSH).

Methods

A correlation exists between blood and salivary levels of MEL.[17] In order to evaluate the basal levels of MEL in the recruited women, MEL was measured in the saliva of all women immediately before the initiation of the trial (late summer 1997).

All women in the study were given a commercial kit for the quantitative determination of MEL in saliva (Diagnos-Techs Inc., Kent, WA). Samples of saliva for measurement of MEL were taken at 2 P.M., 10 P.M., 2 A.M., 8 A.M., and 10 A.M. For testing MEL in saliva, 500 mL of standards, controls, and unknown patient saliva samples were dispensed into labeled tubes. In a second step, fixed amounts of assay buffer, MEL antiserum and ^{125}I-labeled MEL tracer were added to the tubes and incubated for 36–48 h at room temperature. In this reaction the unlabeled MEL of standards, controls, and patients samples and the ^{125}I-labeled MEL compete for the binding sites of the MEL antibody. In a final step, the antibody-bound fraction is precipitated by the addition of a second antibody. Samples were incubated for another 30 min and centrifuged. The supernatant was decanted and the pellet was counted in a Gamma counter. The concentration of MEL of the unknown patient was read from the calibration curve. The sensitivity of the assay is approximately 1.5 pg/mL.

A negative correlation was found between levels of MEL in saliva and age (see below). The correlation between basal MEL levels and menopause was not evaluated.

For determination of hormones in blood, heparinized blood was taken from all fasting women between 8 and 10 A.M., at time 0, 3, and 6 months, irrespective of menstrual cyclicity. The following hormones were assayed by standard laboratory techniques: thyroid hormones (TSH, total T3 and T4), LH, FSH, prolactin (PRL), estrone, estradiol, and progesterone.

Statistics

Results are expressed as mean ± SD. The significance between the means was assessed using paired Student's *t* test. ANOVA test (one-way) was used where appropriate. Correlations were determined by linear regression by the least square method. The differences between the various regression lines were evaluated by analysis of covariance. Chi-square test was used to establish significance of LH increment in MEL-treated women. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Basal Levels of MEL in Saliva

As mentioned above, basal levels of MEL in the saliva were measured in all women before beginning the clinical trial. The women were divided into three groups according to their average nocturnal MEL levels between 10 p.m. and 2 a.m. Low levels were considered those below 20 pg/mL, medium levels were those higher than 20 pg/mL and lower than 300 pg/mL, and high levels those around 300 pg/mL. The age range of the women in our trial was relatively narrow (42–62 years of age), a negative correlation was clearly present between age of the women and basal night levels of MEL ($N = 139$; $r = -0.263$; $P < 0.05$).

Effects of MEL on Blood Levels of Thyroid Hormones

At time 0, before initiation of the trial, a positive correlation was found between basal levels of total T3 and MEL in all women pooled together ($N = 139$; $r = 0.350$; $P < 0.05$). This correlation was absent between MEL and total T4 ($N = 139$;

$r = 0.076$; $P = 0.594$). In the course of the six-month clinical trial, significant changes of thyroid hormone levels were observed in the MEL-treated women. Evening administration of MEL produced a significant increase of total T3 and T4 after 3 and 6 months of MEL administration. When compared to placebo-treated women, the increment of T3 was significant after 3 and 6 months of treatment while T4 only after 6 months.

There were no differences in TSH levels. However, a slightly significant enhancement of T3 was measurable also in placebo-treated women after 6 months of treatment. When thyroid hormone levels were evaluated according to the basal levels of MEL, it was found that the effects of MEL on the increase of T3 and T4 were particularly significant in women with low (below 20 pg/mL) night MEL levels group when T3 and T4 were measured at 3 and 6 months and for the group with the medium (above 20 and below 300 pg/mL) night levels of MEL after six months of treatment. Only T3 was positively modified after six months of treatment in the group of women with high (300 pg/mL) basal levels of MEL. An increase of T3 was also observed in placebo-treatment women after six months of treatment, which was significant in women with high basal levels of MEL. No effects were observed on levels of TSH. MEL administration also produced a significant increment of total T4 in women with low basal level of MEL, when compared to placebo-treated women.

Effects of MEL on Blood Levels

LH

As is well known, progression of aging in women leads to increased blood levels of LH.[18] A consistent increase of basal LH in relation to the increasing age of women was in fact visible at time 0. Also a clear-cut negative correlation between basal levels of MEL and LH ($N = 76$; $r = -0.314$; $P < 0.05$) was found in all women at time 0. When the levels of LH were evaluated in the younger or older age groups (42–49 and 50–62 years of age) in relation to the percentage of women displaying an increment of LH, it was seen that MEL produces a significant decrease in plasma LH only in the younger and not in the older women (see TABLE 1). In fact, in the pla-

TABLE 1. Melatonin effect on LH in perimenopausal and menopausal women

Treatment	Age (years)	N1	N2	%
Melatonin	43–49	38	10	28.6
Placebo	42–49	34	20	61
Melatonin	50–62	30	22	75
Placebo	50–69	38	18	47.6
Melatonin	43–62	71	34	48.6
Placebo	42–59	75	40	53.8

NOTE: Effects of circadian, evening administration of melatonin on LH levels in perimenopausal and menopausal women (42–62 years old) grouped according age. Melatonin produces a more pronounced decrement of LH in the younger women (N1 = number of women taken into consideration; N2 = number of women where the increment of LH after six months is ≥ 10%; %, percent of women where LH increases.

cebo-treated women, no correlation was found between the increment (Δ%) of LH level and the age of the women after six months of treatment, while in MEL-treated women a significant and positive correlation was found between age and LH increment. This demonstrates that the effects of MEL in controlling and maintaining low levels of LH are much more pronounced in the younger women (43–49 years of age) than in the older women (50–62 years of age).

FSH

FSH was measured in all women before initiation of placebo or MEL treatment. As predicted[18] basal levels of FSH increase with age. Similar to LH, a negative correlation exists between basal levels of MEL and FSH at time 0 in all women ($N = 72$; $r = -0.322$; $P < 0.05$). Treatment with MEL for six months produced a significant decrease (Δ%) of FSH especially in women with low basal MEL levels ($N = 70$; $r = 0.468$; $P < 0.05$). After six months of treatment, no correlation was seen between increment of FSH (Δ%) and basal levels of MEL in the placebo-treated women ($N = 74$; $r = -0.057$; $P = 0.795$).

Estrogens, Progesterone, and PRL

The large variability of the values measured in blood samples taken at different times of the menstrual cycle and in menopausal women with age varying from 42 to 62 years of age, prevented the evaluation for significant differences in these hormones between MEL or placebo-treated women within the six-month period of MEL treatment.

Menstrual Cyclicity

Within the relatively short period of MEL administration, only episodical improvement of regularity and duration of menstrual cycles were reported. However, 12 menopausal women (at 1 and 2 years after total cessation of the menses) reported a re-acquisition of normal (bleeding and duration) menstrual cycles.

Other Psychosomatic and Neurovegetative Changes

From the analysis of the completed questionnaire after the initial six months of MEL or placebo treatment, the evaluation of different typical perimenopause or menopause-related symptoms or alterations did not disclose a clear-cut difference between the two groups, with the notable exception of a very significant improvement of mood and complete disappearance of morning depression in the MEL-treated women. Only 6.7% of MEL-treated women reported the continuation of morning depression compared to 21% of placebo-treated women ($P < 0.05$, χ^2-test). Although not significant, many women reported a tendency to amelioration of hot flushes, palpitations, and improvement of quality and duration of sleep.

DISCUSSION

The purpose of this initial trial on possible effects of nocturnal MEL administration in perimenopausal women was to find if MEL by itself modifies levels of hor-

mones and produces changes of any kind, independently of age (42–62 years of age) and the stage of the menstrual cycle. It is undisputed that a close link exists between the pineal gland, MEL, and human reproduction[19] and that a relationship exists between adenohypophyseal and steroid hormones and MEL during the ovarian cycle, perimenopause and menopause.[20]

The preliminary findings emerging from this ongoing clinical trial help to focus and to restrict our attention on the significant changes of thyroid, and of some hypophyseal hormones, namely LH and FSH, after evening administration of exogenous, oral MEL in the course of six months. In fact, no statistically significant changes were observed in the levels gonadal steroids and PRL. This may depend on the different age of women and on the short treatment period (6 months) and because the blood samples were taken with no regard to menstrual cyclicity.

The initial measurement of MEL in the saliva of all women established a criterion for dividing the women in low, medium, and high MEL subjects to see whether or not MEL can produce endocrine changes depending on the basal individual levels of MEL. It was also important to divide all women in two age groups (42–49 and 50–62 years of age) to verify if MEL can affect neuroendocrine functions in relation to the age of the subject. In fact, these separations allowed us to differentiate MEL-reactive and MEL-unreactive women and to disclose an higher sensitivity of younger women to MEL treatment. This is extremely relevant in view of the fact that prevention of perimenopausal and menopause-related endocrine changes may be initiated in women.

The most important and largely unexpected finding was the clear-cut effect of MEL on thyroid function (see TABLE 2). Our trial was initiated in late summer and was concluded in late winter, a season when physiological levels of thyroid hormones are higher.[21] In spite of the obvious variability of T3 and T4 values, when the effect of MEL was evaluated in relation to the basal levels of MEL in the saliva it can be seen that MEL produced a significant increase of T3 and T4 in the low and medium MEL groups, but only T3 increased in the high MEL group. Apparently this effect of MEL on thyroid function can be exerted only when the pineal gland produces less MEL. In the placebo-treated women, endogenous levels of MEL in the group with high MEL seem to affect thyroid function positively. The interpretation of this placebo-dependent change could also be attributed, in the high MEL group, to the physiological changes of thyroid function in relation to temperature and season.[21,22] It is thus clear that low MEL levels can be suggestive of low thyroid function and that administration of MEL may prevent and cure the decline of thyroid function in perimenopausal women. Our findings are confirmed by the positive correlation existing between basal levels of MEL and T3 at time 0 in all women. These results seem also to suggest that thyroid deficiency may be a common and apparently latent endocrine disorder for initiation and progression of menopause in women, and also an unsuspected cause for at least some of the psychosomatic and psychic, neurovegetative symptoms. That MEL can restore deranged thyroid function in perimenopausal women is not a surprise if we consider that MEL modulates the 5′ thyroid deiodinase, that MEL or pineal grafting improve thyroid function in old rodents, and that the pineal gland contains relatively large amounts of TRH. MEL was found to decline significantly from premenopause to postmenopause and to correlate negatively with serum FSH, suggesting that MEL may be determinant for the initiation of menopause.[23]

TABLE 2. Effects of melatonin on thyroid function in perimenopausal and menopausal women

. Melatonin concentration in saliva	T3 (ng/mL)			T4 (ng/mL)			TSH (μg/mL)		
	0 Months	3 Months	6 Months	0 Months	3 Months	6 Months	0 Months	3 Months	6 Months
MEL (*N*=76)	1.44±0.19	1.58± 0.21	1.65±0.22	85.2±8.3	90.2±8.3	94.1±8.6	1.05±0.48	1.03±0.56	1.11±0.65
High (*N*=25)	1.41±0.23	1.56±0.28	1.67±0.33	86.9±9.7	88.9±11	90.2±10.3	1.02±0.39	1.01±0.37	1.24±0.53
Medium (*N*=23)	1.43±0.13	1.44±0.13	1.56±0.08	86.4±6.6	90.5±7.2	95.6±8.7	0.95±0.45	1.04±0.68	1.13±0.64
Low (*N*=25)	1.39±0.20	1.61±0.17	1.60±0.15	84.8±7.1	92.8±4.6	95.4±6.2	1.06±0.39	1.00±0.42	1.14±0.42
Placebo (*N*=78)	1.43±0.20	1.48±0.22	1.54±0.19	85.5±9.2	86.1±11.3	86.4±9.6	1.08±0.54	1.10±0.66	1.16±0.69
High (*N*=24)	1.40±0.14	1.47±0.19	1.71±0.11	85.3±9.1	85.1±9.1	93±9.2	1.09±0.52	1.10±0.30	1.21±0.39
Medium (*N*=28)	1.47±0.22	1.49±0.25	1.55±0.25	86.8±7.4	90.3±11.2	88.2±10.9	0.97±0.38	1.06±0.44	0.99±0.46
Low (*N*=26)	1.39±0.07	1.53±0.23	1.52±0.18	86.4±5.8	85.7±13.7	85.4±8.9	1.05±0.36	1.20±0.45	1.19±0.36

NOTE: Effects of evening administration of melatonin on thyroid function in perimenopausal and menopausal women (42–62 years of age) groups accornding to their basal melatonin levels at night. High = 300 pg/mL, Medium = more than 20 pg/mL and less than 300 pg/mL, Low = less than 20 pg/mL.

FSH may also represent an early endocrine marker of reproductive aging. In our trial the administration of MEL produces significant changes in LH and FSH levels, which seem to be related to the original basal levels of MEL and to the age of the women. MEL depresses the production of FSH only in women with low MEL levels. The apparent partial recovery of more juvenile gonadotropin function in the MEL-treated women is consonant with the notion that progressive increase of LH and FSH in aging women signals an increased central hypothalamic resistance and insensitivity to feedback regulation, with increasing number of LHRH receptors in the hippocampus and compensatory increase of LH and FSH secretion. This effect of MEL in the restoration of reproductive functions is evident in aging experimental animals. Our results indicate that a cause-effect relationship between the decline of nocturnal levels of MEL and onset of menopause may in fact exist. The follow up controls show that MEL abrogates hormonal, menopause-related neurovegetative disturbances and restores menstrual cyclicity and fertility in perimenopausal or menopausal women. At present we can assert that the six-month treatment with MEL produced a remarkable and highly significant improvement of thyroid function, positive changes of gonadotropins towards more juvenile levels, and abrogation of menopause-related depression.

[C*ompeting interests*: The authors declare that they have no competing financial interests.]

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