Biomedicine & Pharmacotherapy 129 (2020) 110485

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Original article

Effect of melatonin administration on the *PER1* and *BMAL1* clock genes in patients with Parkinson's disease

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ARTICLE INFO

Keywords:
Melatonin
Clock genes
*BMAL1*gene
*PER1*gene
Parkinson's disease

ABSTRACT

Sleep disorders are a widespread condition in patients with Parkinson's disease (PD), which has been linked to a deregulation of the circadian cycle and therefore of the clock genes. The aim of this study was to evaluate the effect of melatonin (MEL) on the *PER1* and *BMAL1* clock genes in patients with PD. A double-blind, cross-over, placebo-controlled randomized clinical trial pilot study was conducted in 26 patients with stage 1–3 PD according to the Hoehn & Yahr scale, who received either 25 mg of MEL or a placebo at noon and 30 min before bedtime for three months. The relative expression of the *PER1* and *BMAL1* genes was measured, as well as the presence of daytime, nocturnal, and global sleepiness, and the progression of PD. The levels of the *PER1* and *BMAL1* genes at baseline were 0.9 (0.1–3) *vs.* 0.56 (0.1 − 2.5), respectively; while after the intervention with MEL or placebo the *BMAL1* levels increased to 2.5 (0 − 3.70) *vs.* 2.2 (0.10–3.30), respectively ($d = 0.387$). Fifty percent (50 %) of patients had daytime sleepiness and sixty-five percent (65 %) had abnormal nighttime sleepiness, yet neither group showed changes after the intervention. Patients with PD exhibited an alteration in the levels of the clock genes: MEL increased the levels of *BMAL1*, but the *PER1* levels remained unchanged.

1. Introduction

Parkinson's disease (PD) is the second neurodegenerative disease of the central nervous system, after Alzheimer's disease, characterized by the decrease of dopaminergic neurons in the *substantia nigra pars compacta* [1]. The main motor symptoms in PD are bradykinesia, stiffness, tremor, and postural instability; however, the non-motor symptoms appear early, such as depression, anxiety, hyposmia; and digestive, urinary, and sleep disorders, the latter occurring in approximately 40 % of Mexican patients with PD [2]. Sleep disorders are related to alterations of the circadian rhythm that is a physiological and behavioral cycle regulated by the suprachiasmatic nucleus (SCN); which, through neural and hormonal mechanisms such as melatonin (MEL), sends signals to peripheral clocks throughout the body to synchronize it [3].

At the molecular level, expression of the *BMAL1* and *PER1* clock genes regulates this cycle through negative feedback. The *BMAL1* forms a heterodimer with CLOCK, which allows for transcription of the *PER1*, *PER2*, *CRY1,* and *CRY2* genes when binding to its E-box, and therefore the translation to their respective proteins: *PER* and *CRY* form a dimer, which, when phosphorylated, can enter the nucleus and inhibit the transcription of *BMAL* and *CLOCK* [4]. The *PER1* has a wide oscillation, and *BMAL1* is the central regulator of the biological clock in the SCN. In patients with PD, expression of the clock genes is altered [5,6].

Melatonin is a hormone produced by the pineal gland that regulates the sleep-wake cycle, and at pharmacological doses it is used to reduce sleep disorders. Due to its different mechanisms of action, either through its MT1 and MT2 membrane receptors, or by crossing the cell membrane directly and acting on nuclear receptors [7], it is suggested

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https://doi.org/10.1016/j.biopha.2020.110485
Received 30 March 2020; Received in revised form 18 June 2020; Accepted 30 June 2020

that its use could also normalize the levels of the clock genes expression.

In rats, the administration of rotenone (inhibitor of mitochondrial complex 1, which causes experimental PD) shows a decreased expression of the clock genes that are restored by administering MEL [8]. However, this has not been proven in patients with PD. The aim of the present work was to evaluate the effect of MEL on the expression of the *PER1* and *BMAL1* clock genes, sleep disorders, and disease progression in patients with PD.

2. Material and methods

2.1. Patients

A cross-over, placebo-controlled, double-blind, randomized clinical trial pilot study was carried out in 26 patients with PD treated at the Movement Disorders Clinic (MDC), Department of Neurology, Sub-Specialty Medical Unit, at the Western National Medical Center, of the Mexican Institute of Social Security (known as the *IMSS*) between May and November (summer and early fall). The selected patients had stage 1–3 PD based on the Hoehn & Yahr scale, were more than 20 years old, and agreed to sign the informed consent letter. Excluded, were patients who had movement disorders other than PD, those with previous thalamotomy, pallidotomy or deep brain stimulation; pregnant females, and use of alcohol, coffee, or any antioxidant supplement. The *IMSS* National Scientific Research Committee approved this study with the following number: R-2018-785-019.

A researcher without clinical participation in this study divided the two groups of patients using a random number generator software: MEL-Placebo group and Placebo-MEL group. The first group received 25 mg of MEL at 12 pm and 30 min before bedtime for three months, followed by four days without medication (washout period) and then received 25 mg of placebo at 12 pm and 30 min before bedtime for three months. The second group received 25 mg of placebo at 12 pm and 30 min before bedtime for three months, followed by four days of washout, and then received 25 mg of MEL at 12 pm and 30 min before bedtime for three months. The details in the order of treatment were unknown to the researchers and postgraduate student.

2.2. Melatonin

The MEL and placebo were administered in a pharmaceutical gel-form packet containing water, gelatin, gum, sugar, and inulin as a base; coconut-pineapple as a flavoring; coconut oil as a solubilizer and antioxidant; soy lecithin as an emulsifier; and 25 mg of MEL as an active compound. The company Kurago Biotek®, which provided the gels, concealed the allocation by delivering envelopes containing dark packets to prevent the passage of light, that were the same color and size and labeled with the legend of yellow or green group. A researcher outside the team oversaw delivery of the gel packets to the patients monthly. The researchers were blinded to treatment until the study was complete. Also, an attachment diary was given to the patient, where he/she marked the days when they took the gels and reported if they had any adverse effects.

Melatonin administration schedules were selected because PD patients have low levels of *PER1* and *BMAL1* in the morning and at night, respectively [5,6]. The dosage and timing were based on previous work by our research group, where we gave PD patients the same dose throughout one year and did not observe adverse effects but found positive changes in oxidative stress markers at the third month [9].

The expression of the *PER1* and *BMAL1* clock genes was measured, and, as secondary variables, the presence of sleep disorders and the progression of PD assessed. Measurements were taken at 9 a.m., fasting, and at different times: at the beginning of the study (baseline), at the end of the three months of the first period, at the end of the washout period, and at the end of the three months of the second treatment period. The techniques and instruments used to measure the aforementioned variables are listed below.

2.3. RT-qPCR analysis

To measure the relative expression of the *PER1* and *BMAL1* genes, an RT-qPCR was carried out. Peripheral blood was sampled in Applied Biosystems™ Tempus™ Blood RNA tubes. Total RNA was extracted with the QIAamp RNA Blood Mini Kit. RNA was quantified, and purity was assessed by spectrophotometry. The relative expression was made with the QuantiTect SYBR® Green RT-PCR Kit from Qiagen with the predesigned QuantiTect Primer Assay primers (Hs_PER1_1_SG QT00069265, Hs_ARNTL_1_SG QT00011844, and Hs_GAPDH_1_SG QT00079247) from 10 ng of total RNA according to the supplier's specifications on the Qiagen Rotor-Gene Q equipment. Each sample was run in duplicate, and negative control was introduced per reaction. For the relative expression analysis, the delta-delta method Cq was used.

2.4. Epworth scale

The Epworth scale is an eight-item self-applicable instrument developed to assess the propensity to fall asleep in eight situations, mostly monotonous. A total score of less than 10 was considered normal, 10–12 as indicative of marginal sleepiness, and above 12 suggestive of excessive sleepiness [10].

2.5. SCOPA-Sleep

This is a specific instrument for the evaluation of sleep disorders in patients with PD. It is self-applicable and consists of two sub-scales: the first assesses nighttime sleep (five items: onset, fragmentation, sleep duration, early awakening, and sleep efficiency), and the second assesses daytime sleepiness (six items) during the previous month. A score of higher than seven or five points, respectively, indicates abnormal sleepiness. Additionally, SCOPA-Sleep has a question regarding global sleep evaluation [11,12].

2.6. Unified Parkinson's disease rating scale (UPDRS)

For the longitudinal follow-up of the PD course, the UPDRS was applied through an interview. The scale is composed of 41 items divided by the following domains: Part 1: mental, behavioral, and mood; Part 2: activities of daily living; Part 3: motor evaluation (evaluated by the neurologist); and Part 4: motor complications. The scoring range is from 0 to 199, where "199" represents total disability and "0" represents without disability [13].

2.7. Hoehn and Yahr scale

This scale describes the stage of the patient with PD according to the symptoms, extent of the condition, and physical disability it has caused. It includes five stages assigned from one to five and this was determined by the neurologist.

2.8. Morisky-green

This is a method used to assess patient medication compliance that consists of seven components with dichotomous Yes/No answers and a Likert-type question. A total score of zero translates to high compliance, a score of one or two is equivalent to medium compliance, and a score greater than two corresponds to low compliance [14].

2.9. Statistical analysis and sample size

The sample size was determined using the Rosner formula for the comparison of two means, to detect the increase in the relative

expression of the clock genes from 41 to 47 (SD 7.5), values reported in patients with PD and controls respectively, where the study prepared by Cai Y. et al., was taken as a basis [6]. With a confidence interval (CI) of 95 % and a power of 80 %, a sample size of 24.6 patients was obtained, plus 20 % in losses; and so it was decided to increase the size to 29 patients. To recruit this number of patients an inclusion period of six months was anticipated.

Quantitative variables were expressed as median (minimum and maximum) and the qualitative variables were expressed in frequencies and percentages. The Shapiro Wilk test was used to contrast the normality of the data. The residual effect (baseline measurement *vs.* measurement after washout) and period effect (final measurement of the first period *vs.* final measurement of the second period) were analyzed with the Wilcoxon test for ordinal quantitative or qualitative variables and the McNemar test for qualitative dichotomous variables, as well as the sequence effect (difference of the final measurement of the second period minus the final measurement of the first period between treatments) utilizing the Mann Whitney *U* test. The Mann Whitney *U* test was used to compare the placebo *vs.* MEL effect. To correlate the clock genes with sleep disorders and the progression of PD, the Spearman or Tau c Kendall correlation was used. For all comparisons, a value of $p \leq 0.05$ was considered statistically significant. The magnitude of the effect of the treatments was quantified and converted to Cohen's *d* (standardized mean difference between two independent groups), where a $d > 0.20$ indicates a small effect, $d > 0.50$ indicates a moderate effect and $d > 0.80$ indicates a large effect [15].

3. Results

Of the 150 patients from the MDC who were eligible for the study, 28 were included: 14 began treatment in the MEL-Placebo group, and 14 began in the Placebo-MEL group; nevertheless, in the first group two patients dropped out of the study before the second measurement because their place of residence was distant (Fig. 1). Table 1 shows the clinical and sociodemographic characteristics of the patients: 65 % were male, and the median age was 55 years. Due to the nature of the cross-over study, the intervention was performed in 26 patients, with placebo and MEL, and when crossing it duplicated to 52 comparisons. Before making the comparison between MEL and placebo, the absence of the residual, period, and sequence effects were verified.

3.1. BMAL1 and PER1 clock genes

The *BMAL1* gene expression showed residual and period effects with MEL; therefore, only the first period was taken for analysis (Fig. 2). In the baseline measurement, the levels of the *PER1* gene were higher than those of the *BMAL1* gene; and after the intervention, whether it was with placebo or MEL, the *BMAL1* gene levels increased and the *PER1* levels were maintained. The *BMAL1* expression was similar in patients with MEL and placebo, although with MEL the *BMAL1* levels were three times higher than *PER1,* and with placebo they were twice as high. Table 2.

3.2. Sleep disorders

The baseline measurements in Table 3 show that more than 50 % of the patients had daytime sleepiness and 65 % had abnormal nighttime sleepiness. The patient's perception of sleep in general was, "not well but not badly," but 35 % reported sleeping poorly (Table 2). The presence of abnormal daytime and nighttime sleepiness continued after taking MEL; however, there were fewer patients who reported sleeping poorly after the MEL intervention, but not with placebo.

3.3. PD progression

The final score of parts 1, 3, and 4 of the UPDRS remained similar to baseline measurements. Part 2 (activities of daily living) increased by two points with respect to the baseline measurement in both placebo and MEL. The motor activity of the patients in OFF showed a tendency for a lower score with MEL than placebo ($d = 0.43$) Table 4.

3.4. Correlations

In the baseline measurement, a correlation was found between

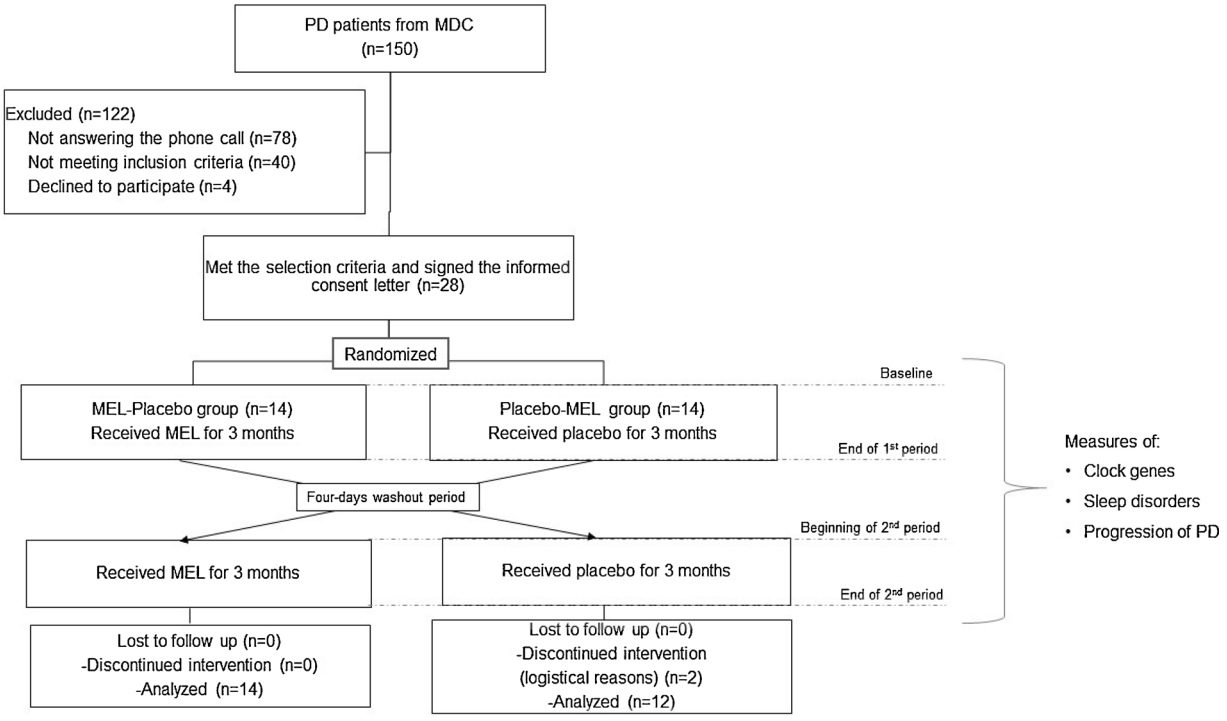

Fig. 1. Flow diagram of the crossed clinical trial phases. Abbreviations: MDC: Movement Disorder Clinic; MEL: Melatonin.

Table 1
Clinical characteristics and baseline demographics.

Variable	Total n = 26	Placebo-MEL n = 14	MEL-Placebo n = 12	*p*
Age (years) *	55. 5 (36 − 71)	57 (38 − 70)	52.5 (36 − 71)	0.66
Male, n (%)[ɬ]	17 (65)	10 (71)	7 (58)	0.68
Weight (kg) *	70.5 (55 − 98)	74 (58 − 91)	63.4 (55 − 98)	0.16
Body Mass Index, n (%)[ɬ]				
Low weight	1 (4)	0	1 (8)	
Normal	12 (46)	6 (43)	6 (50)	
Overweight	8 (31)	6 (43)	4 (34)	0.82
Obesity type 1	5 (19)	2 (14)	1 (8)	
Marital status married, n (%)[ɬ]	19 (73)	7 (50)	12 (100)	0.08
Working, n (%)[ɬ]	11 (42)	6 (43)	5 (42)	0.73
Concomitant diseases, n (%)[ɬ]				
No	16 (61)	10 (71)	6 (50)	
HBP	8 (31)	3 (21)	5 (42)	0.33
HBP + DM2	1 (4)	0	1 (8)	
Hypothyroidism	1 (4)	1 (8)	0	
Years of the evolution of PD*	4.5 (2 − 16)	5.5 (2 − 16)	3.5 (2 − 14)	0.40
Stage of functional disability, n (%)[ɬ]				
1. Unilateral affectation	1 (4)	0	1 (8)	
2. Bilateral affectation with balance	16 (61)	10 (71)	6 (50)	0.37
3. Bilateral affectation without balance	9 (35)	4 (29)	5 (42)	
Number of drugs*	4.5 (0 − 11)	4 (1 − 11)	5 (0 − 8)	0.40
Without pharmacological interaction with MEL, n (%)[ɬ]	7 (65)	9 (64)	8 (67)	1

Abbreviations: HBP: high blood pressure; DM2: diabetes mellitus 2; PD: Parkinson's disease; MEL: melatonin.
Median (minimum-maximum), [ɬ]χ^2, *Mann-Whitney *U* test between Placebo-MEL *vs*. MEL-Placebo.

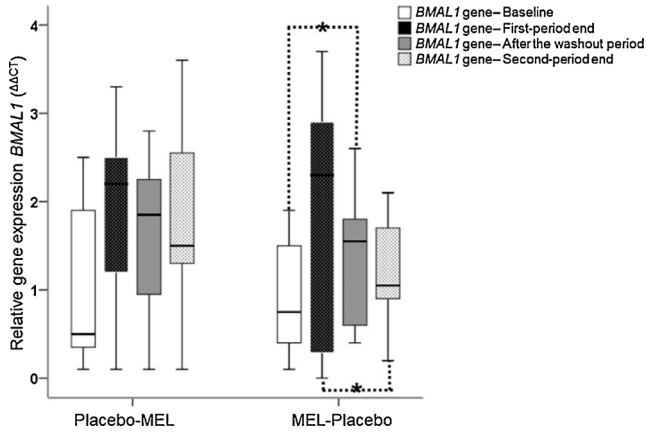

Fig. 2. *BMAL1* residual and period effect by treatment group. Relative *BMAL1* expression continued high after the washout period in the MEL-Placebo group (p = 0.03); once MEL was removed its levels began to decrease (p = 0.05). Abbreviations: MEL: Melatonin.

BMAL1 and age (rho = 0.438, p = 0.03); while at the end of the interventions it was *PER1* that correlated with age (rho = -0.487, p = 0.01). In the MEL group, at the end of the interventions *BMAL1* correlated with daytime sleepiness, both with the EPWORTH scale and the SCOPA-Sleep questionnaire (rho = 0.589, p = 0.04 and rho = 0.674, p = 0.02, respectively).

3.5. Melatonin security

Concerning adverse effects, more than 54 % in both groups did not mention any; while eight patients reported daytime sleepiness, and three patients reported headache, dizziness, nausea, or gastrointestinal problems. The same symptoms were indicated by the placebo group (seven and five, respectively; p = 0.66).

4. Discussion

Expression of the clock genes is directly related to the circadian cycle that regulates various functions such as the sleep-wake cycle. In our study, the baseline levels of relative expression of the *BMAL1* gene around 9:00 am were 57 % lower than the relative expression of the *PER1* gene, which was similar to that reported in PD by Cai Y. et al., and

Table 2
Relative gene expression of clock genes and the frequency of global assessment of nocturnal sleep quality by group. *BMAL1* increased after the intervention, and the perception of the patient's sleep quality improved with MEL compared to the baseline measurement.

	Baseline		Final			
Variable	Placebo n = 14	Melatonin n = 12	Placebo n = 14	Melatonin n = 12	*p*	*d*
Relative expression of clock genes, ($^{\Delta\Delta CT}$)*						
PER1	1.2 (0.2–2.3)	0.8 (0.1–3.0)	1 (0–2.0)	0.8 (0.3 − 6.9)	0.89	−0.31
BMAL1	0.5 (0.1 − 2.5)	0.6 (0.1 − 1.9)[€]	2.2 (0.1 − 3.3)[€]	2.5 (0 − 3.7) [€]	0.63	−0.07
Global sleep quality (SCOPA-S)*, n (%)						
Very well	0	1 (8)	0	1 (8) [€]		
Well	4 (29)	0	6 (43)	3 (25)		
Rather well	0	0	1 (7)	0		
Not well but not badly	6 (43)	6 (50)	3 (21)	6 (50)	0.94	0.09
Rather badly	3 (21)	2 (17)	0	1 (8)		
Badly	1 (7)	3 (25)	4 (29)	1 (8)		
Very badly	0	0	0	0		

Median (minimum-maximum), *Mann-Whitney *U* test between placebo *vs*. MEL, [€]p ≤ 0.05, Wilcoxon signed-rank test between placebo or MEL *vs*. baseline.

Table 3
Sleep disorders by treatment. More than half of patients with PD had sleep disorders.

Variable	Baseline n = 26	Placebo n = 26	Melatonin n = 26	*p*	*d*
Daytime sleepiness (EPWORTH), n (%)*					
Normal	12 (46)	11 (42)	9 (35)		
Marginal	3 (12)	4 (16)	4 (15)	0.55	−0.10
Excessive	11 (42)	11 (42)	13 (50)		
Anormal sleepiness (SCOPA-S), n (%)					
Daytime sleepiness[ɨ]	20 (77)	10 (62)	21 (81)	0.79	−0.04
Nighttime sleep problems[ɨ]	17 (65)	11 (42)	11 (42)	0.65	0.01

Median (minimum-maximum), [ɨ]χ^2, *Mann-Whitney *U* test between placebo *vs*. MEL, [€]$p \leq 0.05$, Wilcoxon signed-rank test between placebo or MEL *vs*. baseline.

Breen D. et al., in humans, and by Mattam U. *et al.,* in rats; probably due to flattened expression of the *BMAL1* gene in patients with PD in the morning; whereas compared to healthy people the *BMAL1* gene is reported as 30 % lower than *PER1* [5,6,8].

Despite the four days of washout in our study, there was a residual effect on *BMAL1* gene expression and in the score of the global sleep evaluation. The NOM-177-SSA1-2013 recommends that washout periods in cross-over designs should be at least seven half-lives of the drug under study [16] (half-life of MEL removal: 45 min), and other cross-over studies with MEL have left only one day as a washout period [17]. However, the high dose of exogenous MEL administered may have caused prolongation in the blood levels, since Gooneratne N. S. et al., explains that this can happen even with much lower doses (4 mg), for up to 10 h later [18].

To our knowledge, this is the first study where MEL is administered to patients with PD and its effect on the expression of clock genes is measured. We found that when administering MEL or placebo, *PER1* tends to be maintained, and that there is a greater tendency in the MEL group for levels of *BMAL1* expression to increase relative to its baseline measurement.

Three mechanisms could have exerted the regularization of the clock genes with the intake of MEL: 1) Joining their MT1 and MT2 membrane receptors that cause the decrease of cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), cAMP response element-binding protein (CREB), and protein kinase C (PKC) and therefore expression of the genes *PER1* and *PER2*; 2) By joining nuclear receptors, such as the orphan RORα receptor, which also positively regulates the transcription of *BMAL1* [19]; 3) Directly inhibiting the proteasome responsible for eliminating the proteins of these clock genes, either by inhibiting the activity of the Ca^{2+}/calmodulin kinase ll (CaMKll) that regulates the phosphorylation of the RPT6 (Sug1) subunit of the regulatory particle of the proteasome, or inhibiting the catalytic center of the proteasome in a covalently reversible way [20]. Therefore, we suggest that administering MEL interferes with the feedback cycles that regulate the transcription of the clock genes. However, when administering MEL at night, when BMAL1 is actively synthesized in the cytoplasm of the cell, it helps stabilize its levels during the day, and when administering MEL during the day, it stabilizes the levels of the PER1 protein during the night.

We observed a strong placebo effect in relation to the expression of *BMAL1*. Quattrone A. et al., report that PD is one of the main clinical disorders for which the placebo response rates are high: more than 50 % of patients show improvement with placebo in motor symptoms [21]. The placebo effect has not been reported on the expression of the clock genes, and the data found suggest that this may be because the placebo promotes dopaminergic activation in the mesolimbic system, allowing the release of dopamine. Dopamine regulates the expression of *CLOCK/BMAL1*: high levels of this will increase the expression of the heterodimer and thus, at the same time, the transcription of the *PER* and *CRY* genes that will be negatively self-regulating this cycle [22–24].

Limitations in our study were the lack of measurements of all the clock genes expressions and of MEL during the day in order to see their behavior or the existence of a phase advance, since *BMAL1* would be expected to be low at night and with MEL have an increase in levels. Nonetheless, it has been reported that patients with PD have a high incidence of sleep disorders (74 %–81 %) related to a disruption of the circadian cycle, where a decrease in the amplitude of MEL secretion is described, and an increase in cortisol levels, flattened expression of the *BMAL1* gene, and lower body temperature are also reported [3,4].

In our study, the majority of patients presented excessive daytime sleepiness and nighttime sleep problems. It is crucial to take into account that the scales used to assess sleepiness and severity reflect the patient's self-perception of sleep, and have high levels of reliability in the population with PD [10,11]. Although the global perception of sleep improved with MEL, nighttime and daytime sleepiness did not show improvements in their scores; which could be due to the annual variation between seasons since in winter motor symptoms tend to increase [25], which also affects the quality of sleep. Also, Zhdanova I. et al., mention that pharmacological doses of MEL (3 − 5 mg) do not increase its sleep-promoting effect and that it might therefore be less effective [26].

The progression of PD tended to increase due to the natural course

Table 4
Progression of Parkinson's disease by treatments. The progression of PD tended to lower the score with the MEL treatment.

Variable	Baseline n = 26	Placebo n = 26	Melatonin n = 26	*p*	*d*
PD progression, (score)*					
1 Mental, behavioral and mood	4 (0 − 8)	4 (1 − 10)	3.5 (2 − 8)	0.84	0.14
2 Activities of daily living	14 (3 − 28)	16 (6 − 27) €	17 (6 − 31) €	0.73	−0.08
3 Motor activity +	25 (15 − 46)	35 (17 − 87)	32 (20 − 53)	0.68	0.28
ON + +	28 (15 − 34)	31 (17 − 41)	29 (22 − 35)	0.71	0.10
OFF + + +	33.5 (20 − 46)	41.5 (22 − 87)	41 (20 − 53)	0.79	0.43
4 Motor complications	9 (0 − 15)	9 (1 − 16)	9 (0 − 27)	0.97	−0.09
Total +	56.5 (33 − 81)	67 (40 − 115)	64 (40 − 92)	1	0.09
ON + +	49 (33 − 81)	67 (45 − 78)	64 (52 − 74)	0.81	0.01
OFF + + +	59 (38 − 79)	64 (40 − 115) €	66 (40 − 92)	0.96	0.18

ON: patients evaluated with the effect of levodopa; OFF: patients evaluated without the effect of levodopa. Abbreviations: PD: Parkinson's disease.
Median (minimum-maximum), *Mann-Whitney *U* test between placebo *vs*. MEL, [€]$p \leq 0.05$, Wilcoxon signed-rank test between placebo or MEL *vs*. baseline; [+]n = 15, [++]n = 7, [+++]n = 8.

of the disease, which is influenced by various factors including the most important: the year in which PD is diagnosed. We observed that the patients in OFF who took MEL had a tendency for a lower motor score (UPDRS 3) than those who took the placebo; since MEL, in addition to being used for the treatment of sleep disorders, is a potent antioxidant and its participation against neurodegeneration has been proposed [9,27–33]. Possibly due to the sample size and the time of treatment with MEL, no difference was found against the placebo for this variable.

In the *BMAL1* baseline measurement it was correlated to age, but after the administration of MEL it disappeared, and a negative relationship was found between *PER1* and age. The *BMAL1* is associated with premature aging and reduced life span [34], and probably, when administering MEL, due to its antioxidant effect this association disappeared.

Finally, it has been reported that *PER1* decreases as age increases [34], and because the *PER1* levels were maintained this correlation appeared in this study. Regarding the correlation between *BMAL1* and daytime sleepiness, Von Schantz et al., comment that a mutation of the *PER2* gene causes its hypophosphorylation [35,36] and is related to daytime sleepiness; *PER2* without phosphorylation cannot enter the nucleus to exert its inhibitory function in *BMAL1*; and consequently, we believe that this could promote daytime sleepiness in addition to the high levels of *BMAL1*.

The results of this study only explain a part of the regulation of the circadian cycle since there are other factors such as light, food, physical activity, or depression and anxiety [37] that are involved in the regulation of the circadian cycle and should be taken into account in future research. Our research team is already working on a protocol with longer exposure time to exogenous MEL (18 months) but with a single and lower dose on a different administration schedule, including the measurement of the *CRY* and *CLOCK* genes.

5. Conclusion

This is the first study in patients with PD where the effects of MEL on the expression of clock genes was examined. We found an alteration in the *PER1:BMAL1* relationship in patients with PD. By administering MEL for three months, *BMAL1* levels increased in the morning, like they did with placebo, but MEL was more effective at increasing *BMAL1* levels. Furthermore, the use of high-dose MEL failed to reduce the presence of insomnia or excessive daytime sleepiness, but it increased the global perception of sleep comfort. The PD progression score increased according to its natural course, regardless of the intervention; while OFF patients who consumed MEL tended to have improved motor symptoms.

Funding

This work was supported by the Kurago Biotek® company.

Author contributions

All authors listed have made a substantial direct and intellectual contribution to the work and have approved it for publication.

Declaration of Competing Interest

None.

Acknowledgments

To the Mexican National Council of Science and Technology (CONACYT) and Mexican Institute of Social Security for the scholarship granted to Daniela LC Delgado-Lara, and to the company Kurago Biotek® for their support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.110485.

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