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Impact of Melatonin on Motor, Cognitive and Neuroimaging Indices in Patients with Multiple Sclerosis

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ABSTRACT

A series of preclinical and clinical studies have shown the immunomodulatory effect of melatonin, especially in the state of chronic inflammation.

A double-blind, randomized, parallel-group, placebo-controlled clinical trial was designed to study the tolerability and efficacy of supplemental therapy with melatonin (3 mg/day) in comparison to placebo in relapsing-remitting MS (RRMS) patients receiving once weekly interferon beta. Patients were followed up for 12 months. Primary outcomes consisted of the number of relapses, change in Extended Disability Status Scale (EDSS), and the number and volume of new T2 and gadolinium-enhancing brain lesions. Secondary outcomes included change in performance on Multiple Sclerosis Functional Composite (MSFC) as well as change in fatigue and depression. The outcomes were evaluated every three months.

Twenty-six patients (13 in each group) were recruited in the study. All participants, except for one patient in the placebo group, completed the study. No patient reported serious adverse events. There was no significant difference either in primary or secondary outcomes between melatonin and placebo arm. However, a trend for beneficial effect was observed for melatonin on change in MSFC performance and the cognitive subscore of the Modified Fatigue Impact Scale (p=0.05 and 0.006, respectively, not corrected for multiple comparisons).

We found no significant effect for treatment with melatonin on measures of clinical and functional disability and development of brain lesions in our small sample-size study. Studies with higher statistical power and longer follow up are needed to further evaluate the potential immunomodulatory effect of melatonin in RRMS treatment.

Keywords: Immunomodulation; Melatonin; Multiple sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is the most frequent chronic inflammatory disease of the central nervous system that

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overactivity of autoreactive immune system as well as oxidative stress play a cardinal role in neurodegeneration and axonal loss in it. There are some diseases modifying drugs (DMDs) for this potentially disabling disease but with only partial efficacy in disease course. Therefore during recent years several clinical trials have been designed to add other immunomodulatory drugs in order to increase the efficacy of prior DMDs.

Melatonin (N-acetyl-5-methoxy-tryptamine) is synthesized from amino acid tryptophan.[1] Release of melatonin exhibits a circadian pattern with a maximal peak at darkness and minimum levels during lightness.[2] It has been well known that pineal gland via melatonin signal pathways plays important role in modulating several physiological functions including regulation of biological clock, immune mediators and cytokines, sex hormones, opioids, and glucocorticoids.[3-5] A series of preclinical and clinical studies have shown that pineal melatonin hormone plays an important role in modulating immune response.[6-7] In some circumstances, it exhibits pro-inflammatory effects, while in hypoxic states and chronic inflammation, melatonin acts as an anti-inflammatory and neuroprotective agent.[9-12] The precise mechanism through which melatonin modulates immune system, is still unclear. It has been suggested that melatonin behaves as an immune buffer so that in immunocompromised conditions, it acts as an immunostimulant and in immune system overactivity, melatonin shows anti-inflammatory and anti oxidant properties.[11] Regarding the above evidences it has been proposed that melatonin may modulate immune response in MS. Therefore, we designed a double-blind randomized clinical trial to evaluate efficacy of melatonin compared to placebo in patients with relapsing-remitting multiple sclerosis (RRMS). Herein, we monitored patients for motor and cognitive functions and brain radiological alterations for a 12-months period.

MATERIALS AND METHODS

Ethics Approval

This trial was registered at ClinicalTrials.gov (Identifier: NCT01279876). It was conducted in accordance with the Declaration of Helsinki, and was approved by the ethics review board of Tehran University of Medical Sciences. All patients provided written informed consent before participation in the study.

Participants

Participants were limited to patients referring to MS clinics of Tehran University of Medical Sciences at Imam Khomeini and Sina hospitals. Patients diagnosed with RRMS based on the 2005 McDonald criteria[13] who were receiving once weekly interferon beta, had no history of other chronic systemic medical illnesses and met the following inclusion criteria were recruited in the study: (i) age 18-55 years, (ii) Expanded Disability Status Scale[14] (EDSS) number <4, (iii) disease duration >6 months, (iv) consumption of once weekly interferon beta for at least 6 months. Patients were excluded if they had a history of steroid use or relapse in the 12 months prior to enrollment in the study or were under treatment with drugs that affect melatonin metabolism.

Study Design and Outcomes

The study was a double blind, randomized, parallel-group, placebo-controlled clinical trial. Using a randomized block design, patients were assigned to receive melatonin (3mg, hora somni) or placebo for 12 months, while they continued their treatment with once weekly interferon beta. Patients were examined by a neurologist for EDSS and Multiple Sclerosis Functional Composite[15] (MSFC) scoring, completed Modified Fatigue Impact Scale[16] (MFIS) and Beck Depression Inventory-second edition[17] (BDI-II) questionnaires, and underwent brain MRI at the beginning and end of the study. They were visited every 3 months during the follow-up period, so that their history of relapse, experience of drug adverse effects, and EDSS numbers were recorded quarterly.

The number of relapses and change in EDSS number were designated as safety issues, and patients who had an increase of more than 1 in their EDSS numbers or had two or more relapses during the 1-year follow-up period were to be excluded from the study. These measures, along with the number and volume of gadolinium-enhancing and new T2 lesions were the primary outcome measures of the study. Change in MSFC, MFIS and BDI-II scores were considered as secondary outcomes.

Magnetic Resonance Imaging

Imaging was performed using a GE Signa 1.5T MRI scanner (General Electric Medical Systems, Milwaukee, WI). Whole-brain MRI protocol at the baseline consisted of: (i) Axial high-resolution T1-weighted 3D IR-SPGR (TR/TE=13/4 ms, TI=400ms, slice thickness=1.2 mm, gap=0 mm), (ii) Axial T2-weighted FSE (TR/TE=6000/100 ms, slice thickness=1.5 mm, gap=0 mm), (iii) Axial PD-weighted FSE (TR/TE=3000/20 ms, slice thickness=1.5 mm, gap=0 mm) (iv) Axial FLAIR FSE (TR/TE/TI=7000/100/2200 ms, slice thickness=5 mm, gap=0 mm). The follow-up imaging protocol was the same as baseline, except for an additional post-gadolinium axial T1-weighted FSE image (TR/TE=660/20 ms, slice thickness=1.5 mm, gap=0 mm) obtained 10 minutes after gadolinium injection.

Lesion Segmentation

Each patient's baseline T2-weighted scan was used as a reference for image registration. Baseline PD and FLAIR images, in addition to follow-up T2, PD, FLAIR and post-gadolinium images were registered to the baseline T2 scan using rigid-body transformation implemented in FLIRT,[18] and then underwent noise reduction using SUSAN.[19] A neuroradiologist, who was blind to the patients' treatment groups, segmented baseline T2 lesions along with follow-up gadolinium-enhancing and new T2 lesions using a semi-automatic manual tracing pipeline available in Amira (Visage Imaging GmbH, www.amira.com) according to an MRI atlas of MS lesions.

Statistical Analysis

All analyses assumed a two-sided test of hypothesis with a significance level of 0.05. Nonparametric tests and general linear model were used to compare between groups. Mann-Whitney U test was used for pair wise comparison of continuous variables, and Chi-square test was used to assess differences in categorical data. General linear model was used to assess the effect of treatment on change in outcome measures while accounting for age and sex.

RESULTS

Twenty-six patients (13 cases in each treatment group) were recruited in this study from 2010 to 2013. Twenty-five patients, including all the patients in the melatonin group, completed the study. One patient recruited in the placebo group left the study before completion due to personal reasons. Baseline characteristics of the participants are summarized in Table 1.

Table 1. Baseline characteristics of treatment groups

Characteristics at baseline	Melatonin group (N=13)	Placebo group (N=12)	*p*-value
Age[a], yr (Mean ±SD)	33.3 (±7.6)	34.5 (±8.2)	0.70
Gender[b], F/M	9/4	12/0	0.096*
Disease duration[a], yr (Mean ±SD)	6 (±4.7)	2.8 (±1.7)	0.08*
Education[a], yr (Mean ±SD)	13.9 (±3.0)	13.3 (±2.8)	0.59
Age at onset[a], yr (Mean ±SD)	27.2 (±6.7)	31.7 (±8.6)	0.21
EDSS[c] (Mean ±SD)	1.8 (±1.5)	0.77 (±1.0)	0.09*
MSFC[a] (Mean ±SD)	0.1 (±0.9)	-0.1 (±0.5)	0.48
MFIS[a] (Mean ±SD)	38.3 (±25.7)	29.7 (±12.6)	0.46
BDI-II[a] (Mean ±SD)	19.2 (±10.7)	18.6 (±9.9)	0.93
T2 lesion volume[a], mm^3 (Mean ±SD)	5049 (±5434)	4094 (±4144)	0.77

Abbreviations: BDI-II=Beck Depression Inventory-Second edition, EDSS=Expanded Disability Status Scale, F=female, M= male, MFIS=Modified Fatigue Impact Scale, MSFC=Multiple Sclerosis Functional Composite, SD= standard deviation. (* $p<0.1$).

[a] Mann-Whitney U test

[b] Fisher's Exact Test

[c] Pearson Chi-Square

Table 2. The effect of treatment on primary outcome measures of the study.

Primary endpoints	Melatonin group	Placebo group	*p*-value
Patients with no relapse[a]	10	9	0.91
Number of Relapses[a]	3	3	0.55
Patients with improved or stable EDSS[a]	8	10	0.59
Mean change in EDSS[b] (±SD)	0.09 (±0.3)	0.05 (±0.15)	0.20
Patients with no new T2 lesion[a]	4	5	0.96
New T2 lesion number[c] (±SD)	0.8 (±0.8)	0.7 (±0.9)	0.33
New T2 lesion volume[c], mm^3 (±SD)	53 (±93)	104 (±211)	0.78
Patients with no Gd-enhancing lesion[a]	7	9	0.82
Gd-enhancing lesion number[c](±SD)	0.3 (±0.7)	0.2 (±0.4)	0.50
Gd-enhancing lesion volume[c], mm^3 (±SD)	7 (±16)	12 (±36)	0.91

Abbreviations: EDSS=Expanded Disability Status Scale, Gd=gadolinium, SD=standard deviation.

[a] Pearson Chi-Square

[b] General linear model with the difference between follow-up and baseline measures as dependent variable, and treatment group, age, and gender as predictors. P-value for treatment group is reported.

[c] Mann-Whitney U test

No significant difference was detected between the baseline demographic and clinical characteristics of patients recruited in the two treatment groups. However, a trend was observed towards recruitment of a higher number of male patients, and patients with longer disease duration and higher EDSS numbers in the melatonin group (*p*=0.096, 0.08, and 0.09, respectively).

None of the patients in the melatonin group reported serious adverse events such as daytime somnolence, headache and dizziness. No patient was excluded because of safety issues (two or more relapses or an increase of more than 1 in EDSS number). None of the primary outcome measures, including the number of relapses (*p*=0.55), change in EDSS (*p*=0.20), and the number and volume of gadolinium-enhancing (*p*=0.5 and 0.91, respectively) and new T2 lesions (*p*=0.33 and 0.78, respectively), showed significant difference between treatment with melatonin and placebo (Table 2).

Moreover, we did not detect any significant difference between changes in BDI_II scores in the two treatment groups (*p*=0.76). Although not significant after correcting for multiple comparisons, a positive trend was observed for the effect of melatonin on change in MSFC and MFIS numbers (*p*=0.05 and 0.098, respectively) (Table 3). Patients recruited in the melatonin group tended to show improved performance on MSFC compared to baseline (mean±SD:-0.12±0.26), while the performance of participants in the placebo group tended to have been deteriorated (mean±SD:0.13 ±0.52). MFIS numbers tended to decrease more in the melatonin group (mean±SD:-11.8 ±17.9) in comparison to the placebo group (mean±SD:-3.9±15.8). Post-hoc analysis on the sub-tests of MSFC and the subscores of MFIS revealed a trend for the effect of melatonin on performance in 9-Hole Peg Test (*p*=0.06) and the MFIS cognitive subscore (*p*=0.006).

Summary and Interpretation of Results

No beneficial effect was observed in melatonin group compared to placebo group in this project. No meaningful difference was detected in evaluated endpoints. As expected, most of patients reported that satisfying changes had experienced in quality and duration of their sleep. Other than one patient in placebo group, all patients could tolerate the drug and complete the study. Overall, this study showed that melatonin has no immunomodulatory effect in multiple sclerosis.

Table 3. The effect of treatment on secondary outcome measures of the study. The comparison is made using general linear model, with the difference between follow-up and baseline measures as dependent variable, and treatment group, age, and gender as predictors. *P*-value for treatment group is reported.

Secondary endpoints	Melatonin group	Placebo group	*p*-value
Mean change in MSFC (±SD)	-0.12 (±0.26)	0.13 (±0.52)	0.05
25FW	-0.3 (±0.52)	0.02 (±0.82)	0.79
9HPT	0.0004 (±0.003)	0.003 (±0.005)	0.06
PASAT-3"	3.7 (±6.6)	7 (±10)	0.33
PASAT-2"	1.4 (±10)	3.7 (±4.7)	0.81
Mean change in MFIS (±SD)	-11.8 (±17.9)	-3.9 (±15.8)	0.098
Physical subscore	-4.8 (±8)	-3.4 (±10.8)	0.45
Cognitive subscore	-5.8 (±8)	-0.8 (±5.9)	0.006
Psychosocial subscore	-1.2 (±2.5)	-1.3 (±2.7)	0.72
Mean change in BDI-II (±SD)	-0.08 (±8.6)	-4.3 (±7.8)	0.76

Abbreviations: 25FW=Timed 25-Foot Walk Test, 9HPT=9-Hole Peg Test, BDI-II= Beck Depression Inventory-Second edition, MFIS=Modified Fatigue Impact Scale, MSFC=Multiple Sclerosis Functional Composite, PASAT= Paced Auditory Serial Addition Test, SD=standard deviation. Bold font: $p<0.1^{a}$ Pearson Chi-Square

DISCUSSION

Melatonin has been shown to have paradoxical pro-inflammatory and anti-inflammatory properties. Pleiotropic effects of melatonin results from different mechanism of action, via binding to high-affinity membrane G-protein-coupled receptors and/or intracellular receptors, and is dependent to the stage of inflammation.[11,20] Melatonin exhibits pro-inflammatory effect by upregulation of major histocompatibility complex-II (MHC-II) expression and increase in Th1-related cytokines through activation of surface melatonin receptor on antigen presenting cells.[9-10,21-22] On the other side, melatonin has been shown to have impact neuroprotective and anti-oxidant properties through interaction with intracellular receptors in hypoxic state and chronic inflammation.[11-12, 22] It is also believed that lymphocytes, like some other tissues, present melatonin receptor and could secrete melatonin that probably in an autocrine/paracrine manner affects immune cells.[11,23]

Since last decade, melatonin has been used for managing sleep disorders in MS patients.[24] However, the importance of melatonin alterations in MS pathogenesis is not fully recognized. Several experimental studies implicated that melatonin alterations may be contributed in the pathogenesis of multiple sclerosis. Jankovic et al. showed that early pinealectomy predisposes rates to EAE, while late pinealectomy (after 6 weeks age) could be protective against induction of EAE.[25-26] This connotes that melatonin must play important role in modulating autoactive T cells in early life. Previously, in a case-control study in Iranian population of MS, Gholipour et al. reported that the urinary level of melatonin decreased in MS patients compared to control group.[27] Likewise; there was a meaningful correlation between melatonin level and MSFC. Thereupon, based on neuroprotective and immunomodulatory effects of melatonin in chronic inflammation demonstrated by preclinical and clinical observations, it has been suggested that melatonin may be effective in modulating immune response in MS.[11] Recently, it has been shown that melatonin has antioxidant properties in MS patients.[28-30]

Despite theoretical hypothesis, no beneficial effect has been observed in melatonin group compared to placebo group in this project. No meaningful difference was detected in evaluated endpoints. As expected, most of patients reported that satisfying changes had experienced in quality and duration of their sleep. Other than one patient who was excluded because of hypersomnolence, all patients could tolerate the drug and complete the study.

However, this should be interpreted cautiously. Our sample size was small which has not resulted in a perfect randomization of subjects, as suggested with the *p*-values<0.1 in Table 1. Likewise, low sample size

means low power for finding significant results. Short follow-up duration (one-year) and our measures may not be sensitive enough for evaluating immunomodulatory and neuroprotective effect of melatonin in such sample size. Another confidential problem could be relatively stable disease state of our patients before starting the clinical trial. It is usual to consider at least one relapse during the year before study for finding more reliable results.

In summary, no immunomodulatory effect has been observed in melatonin group compared to placebo group over a period of 12 months. Nonetheless, this study showed that adding melatonin to once weekly interferon beta in MS patients is well tolerated without any serious side effect.

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