Evidence for possible role of melatonin in reducing oxidative stress in multiple sclerosis through its effect on SIRT1 and antioxidant enzymes

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A B S T R A C T

Aims: Oxidative stress plays a crucial role in the pathogenesis of multiple sclerosis (MS). Melatonin has a central role in the modulation of oxidative stress pathways. We aimed to investigate the effect of melatonin on mRNA expression and activity of sirtuin1 (SIRT1) and its target genes, manganese superoxide dismutase (MnSOD), and catalase in peripheral blood mononuclear cells (PBMCs) from MS patients and healthy subjects.

Key methods: This study was performed on 12 patients with relapsing–remitting MS (RRMS) and 14 age- and sex-matched healthy subjects. PBMCs were isolated and treated with pharmacological concentration of melatonin (1 mM) for 12 h. Gene expression was evaluated by real-time PCR. SIRT1 activity in PBMCs was measured using a fluorometric assay. MnSOD and catalase activities in PBMCs were determined by colorimetric assays. Plasma total antioxidant capacity was measured using the ferric reducing ability of plasma assay.

Key findings: Melatonin significantly increased activities and mRNA levels of SIRT1 and catalase in both patients and healthy subjects, whereas melatonin treatment caused a pronounced increase in MnSOD mRNA expression and activity only in patients. In MS patients, SIRT1 activity did not correlate with catalase and MnSOD activities before melatonin treatment; while a significant correlation was observed between SIRT1 activity and catalase activity in PBMCs of patients after melatonin treatment.

Significance: It appears that the antioxidant status is affected in PBMCs from MS patients and melatonin could improve impaired antioxidant defense in MS through upregulation of SIRT1, MnSOD and catalase, which might be important in MS management.

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a multifunctional molecule that, among its broad range of functions, modulates oxidative stress pathways [23–25], thus making it particularly interesting for MS therapy. Based on available literature, melatonin acts as a potent antioxidant through direct free radical scavenging properties, as well as by its role in inducing the expression and activities of several antioxidant enzymes [23,25]. Evidence from recent clinical studies indicates an association between dysregulation of melatonin and MS [26–29]. It has been also suggested that melatonin supplementation may be a consideration for MS management [30–32]. However the underlying molecular mechanisms have not been fully elucidated.

Peripheral blood mononuclear cells (PBMCs) were shown to be helpful in better understanding the complex nature of the disease, and used in monitoring the response to treatment [33]. There is evidence that PBMCs are helpful tools to study the status of antioxidant defense system [34]. To date, no study has investigated the antioxidant properties of melatonin on PBMCs. Moreover, little is known about the protective effects of melatonin in the context of MS. Therefore, we aimed to investigate and compare the effect of melatonin treatment on gene expression and activity of SIRT1 and its target genes, MnSOD, and catalase in PBMCs of RRMS patients and healthy subjects.

2. Materials and methods

2.1. Patients and healthy subjects

From April, 2014 to June, 2014, patients with RRMS (n = 12) and age-matched healthy subjects (n = 14) participated in this study. All participants (patients and healthy subjects) were women between the ages of 20–40 years.

Patients with RRMS were consecutively recruited from Sina MS Research Center, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran. All patients had a clinically definite diagnosis of MS based on the McDonald criteria (2010) [3]. It should be noted that all patients had a first-time diagnosis of RRMS at time of sampling.

Patients had never received any MS-related medication (e.g., interferon-β) or glatiramer acetate corticosteroid therapy and other currently used immunomodulatory drugs prior to sampling. All patients were in relapse phase of MS. Relapse was defined as patient-reported symptoms or objectively observed signs typical of an acute inflammatory demyelinating event in the CNS, persisting for at least 24 h, in the absence of fever or infection [3].

A magnetic resonance imaging (MRI) examination and laboratory experiments including IgG index, and oligoclonal IgG bands were carried out in all RRMS patients prior to the study. In all patients, the neurological examinations, clinical assessments and MS diagnosis were made by an expert neurologist. Finally, MS diagnosis was confirmed by at least two other independent neurologists.

The level of disability was assessed using the Expanded Disability Status Scale (EDSS). We recruited RRMS patients who had EDSS between 1 and 6. In our study, the mean EDSS was 3.7.

It should be noted that the differential diagnosis for clinically isolated syndrome (CIS), neuromyelitis optica (NMO) (also known as Devic’s disease), and vasculitis and vitamin B12 deficiency was performed and patients suspected of having CIS, NMO, vasculitis and vitamin B12 deficiency were excluded from study.

Healthy women as the control group were selected from the same geographical areas and did not have any clinical and laboratory characteristics of autoimmune diseases and neurological disorders. Healthy subjects had no family history of MS and other autoimmune diseases. All healthy subjects were received a regular medical check-up by a physician.

The other exclusion criteria for all participants (patients and healthy subjects) were as follows: 1) treated with antioxidant supplements, anti-inflammatory drugs, and vitamins in the previous 6 months; 2) had a history of acute or chronic infection, malignancy, diabetes (types I and II), and any clinically significant systemic disease; 3) was currently pregnant or lactating; 4) was currently smoking; and 5) used contraceptive medication during the previous 6 months.

This study was approved by the Ethics Committee of Tehran University of Medical Sciences, and written informed consent was obtained from all participants prior to the study entry.

2.2. Cell culture

Peripheral blood samples were collected in Vacutainer tubes containing sodium heparin following overnight fasting between 7:00 a.m. and 8:00 a.m. PBMCs were isolated from heparinized blood samples using Ficoll– Hypaque (Lympholyte-H; Cedarlane Laboratories, Hornby, ON, Canada) gradients centrifugation.

The PBMC layer was collected, washed twice with sterile phosphate-buffered saline (GIBCO; Invitrogen Laboratories, UK) and suspended at 2 × 10⁶ cells/well and 4 × 10⁶ cells/well in 12- and 6-well flat-bottom plates, respectively, containing RPMI 1640 GlutaMAX medium (GIBCO; Invitrogen Laboratories, UK) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO; Invitrogen Laboratories, UK), and 1% penicillin/streptomycin solution (GIBCO; Invitrogen Laboratories, UK). After overnight incubation at 37 °C with 5% CO2, cells were treated as follows: control (only with 0.1% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) and melatonin (1 mM) (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 12 h at 37 °C in an atmosphere of 5% CO2. Finally, the cells were collected and stored at −80 °C for further experiments. It should be noted that melatonin was dissolved in DMSO and diluted in RPMI1640 before use. The final concentration of DMSO in the culture medium was 0.1%.

2.3. Evaluation of cell viability under melatonin treatment

The cell viability was determined using MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide) (Sigma–Aldrich, St. Louis, MO, USA) colorimetric assay, which reflects mitochondrial dehydrogenase activity in viable cells.

Briefly, PBMCs (at density 25 × 10⁴/well) were seeded in 96-well plates and cultured in medium containing different concentration of melatonin (1 nM, 1 μM and 1 mM) and incubated for 12 h at 37 °C in the humidified atmosphere with 5% CO2. Then, PBMCs were washed three times with phosphate-buffered saline and the MTT labeling reagent (500 μg/ml) was added into wells and PBMCs were further incubated for 4 h at 37 °C. The media was discarded and the blue formazan crystals were solubilized with DMSO and optical density was measured at 540 nm. It should be noted that in addition to MTT assay, cell viability was also assessed by trypsin blue exclusion method and calculated by the following formula: percent cell viability = 100 × (number of viable cells) / (total number of cells).

2.4. Quantitative real-time PCR

Total RNA was extracted from PBMCs (2 × 10⁶ cells/well) using a Total RNA Extraction Miniprep kit (Viogene, Taiwan) following the manufacturer’s instruction. RNA was quantified spectrophotometrically by measuring the absorbance at 260 nm and RNA purity was determined by determining the 260/280 nm absorbance ratio. RNA integrity was evaluated by agarose gel electrophoresis. One microgram of DNase-treated RNA was reverse transcribed into complementary DNA (cDNA) using Revert Aid First Strand cDNA Synthesis kit (Thermo Scientific, Fermentas, USA).

Real-time PCR was performed in a Rotor Gene real-time thermocycler (Qiagen, Hilden, Germany) using SYBR Green detection kit (Takara Bio, Ostu, Japan). Primers for target genes (SIRT1, MnSOD and catalase) and housekeeping gene β-actin were purchased from Qiagen (Hilden, Germany). It should be noted that the linear range of the real-time PCR was determined by generating a standard curve for β-actin, SIRT1, MnSOD, and catalase prior to performing the assay on...
test samples. Specifically, a cDNA pool was serially diluted (472.5–5.8 ng in 3-fold dilution) to generate a standard curve. The correlation coefficients of all the standard curves showed good linearity ($r^2 > 0.95$), and all experiment samples were confirmed to fall within this range. The specificity of amplification was verified by analyzing their characteristic melting curve and by subsequent gel electrophoresis. Relative gene expression was normalized to β-actin and calculated as $2^{-\Delta\Delta CT}$ using the formula: $2^{-\Delta(CT\ target\ gene-CT\ \beta-actin)}$.

2.5. Assessment of plasma total antioxidant capacity (TAC)

Plasma TAC was determined using the ferric reducing ability of plasma (FRAP) assay described by Benzie and Strain [35]. This method is based on the ability of plasma to reduce a colorless ferric tripyridyltriazine complex (Fe$^{3+}$-TPTZ) (Sigma-Aldrich, St. Louis, MO, USA) to the blue colored ferrous-tripyridyltriazine (Fe$^{2+}$-TPTZ) complex at low pH, which can be obtained by comparing the absorbance change at 593 nm in test samples with standard solutions containing known concentration of ferrous ions.

2.6. The assessment of MnSOD activity

MnSOD activity in PBMCs (2 × 10$^6$ cells/well) was determined using a commercial available colorimetric assay (SOD activity Enzo Life Sciences; USA). The procedure is based on the Mn-SOD's ability to inhibit the reduction of WST-1 (water soluble tetrazolium salt) to WST-1 formazan by neutralizing superoxide anions produced by the xanthine/xanthine oxidase system. MnSOD activity was assessed by preincubating the cell lysate with 2 mM potassium cyanide (KCN) which inactivates both Cu/Zn-SOD and extracellular SOD. Prior to measurement of MnSOD activity, the linear range of the assay was determined by serially diluting cell extracts with 1 × SOD buffer with concentrations ranging between 0.5 μg/25 μl and 50 μg/25 μl and comparing the percentage of inhibition of the rate of formation of WST-1 formazan with a standard curve generated by serial dilutions of the SOD standard. The SOD standard, WST-1 reagent, and 1 × SOD buffer were supplied in kit form. Protein concentration was determined using Bradford method [36]. MnSOD activity data were expressed as units/mg of protein.

2.7. The assessment of catalase activity

Catalase activity in PBMCs (2 × 10$^6$ cells/well) was measured using the catalase assay kit (Ab83464; Abcam, Cambridge, MA, USA) according to the manufacturer’s instructions. The procedure is based on the function of catalase in decomposing hydrogen peroxide (H$_2$O$_2$) to water and oxygen in the presence of an optimal concentration of H$_2$O$_2$. The unconverted H$_2$O$_2$ reacts with OxiRed probe to produce a product that can be measured spectrophotometrically at 570 nm. Briefly, harvested cells were lysed in a cold assay buffer (provided with kit) and sonication on ice. Following centrifugation, the supernatant was collected and the protein concentration of the resulting cell lysate was determined using the Bradford assay. The catalase activity in the PBMCs was reported as units/mg of protein.

2.8. The measurement of SIRT1 activity

SIRT1 activity was determined in PBMCs (4 × 10$^6$ cells/well) by the Cyclex SIRT1/Sir2 Deacetylase Fluorometric Assay Kit (Cyclex, Nagano, Japan). This kit uses the NAD-dependent deacetylase activity of SIRT1 on the specific substrate, in the presence of trichostatin A, a potent inhibitor of SIRT1-independent histone deacetylases. The nuclear and cytoplasmic extracts were prepared from harvested PBMCs using an EpiSeeker Nuclear Extraction Kit (Abcam) according to the manufacturer’s instructions. Fluorescence intensity (excitation 340 nm, emission 440 nm) was read using a microplate fluorometer (Synergy H4 Bio Tek) every 1 min for 1 h and the fluorescence intensity change [arbitrary fluorescence unit (AFU)] per minute was normalized to the protein concentration.

2.9. Statistical analysis

All data were analyzed using SPSS 19 (SPSS Inc., Chicago, IL, USA) and presented as mean ± standard error of the mean (SEM). Comparisons between groups (RR-MS patients and healthy subjects) and within group were carried out by the Student’s independent t test and the paired Student’s t test, respectively. Comparative CT method [37] was used for analysis of the gene expression. Statistical significance was considered at a p-value < 0.05, unless noted.

3. Results

A total of 12 RRMS patients (all women) and 14 healthy subjects (all women), were selected for this study. The demographic features of the RRMS patients and control subjects and the initial symptoms and location of the CNS lesions in RRMS patients are presented in Table 1. There was no statistically significant difference between patients and control subjects with regard to age and body mass index. In our study, RRMS patients had a mean EDSS of 3.7 (range, 1–6). As shown in Table 1, five patients (42%) had signs of cranial nerve involvement, as initial symptoms. Motor symptoms were observed in four patients (33%) as first symptoms and sensory symptoms were recorded in three patients (25%) as presenting symptoms. Accordingly, all patients were monosymptomatic.

Results from the brain MRI scans demonstrated that all of the RRMS patients had at least four periventricular lesions. As shown in Table 1, juxtacortical lesions were observed in 9 (75%) patients and infratentorial lesions were seen in 7 (58%) patients. No patients had evidence of spinal cord lesions. In details, four patients had juxtacortical, infratentorial and periventricular lesions. Five patients had both juxtacortical lesions and periventricular lesions and three patients had both infratentorial and periventricular lesions. BMI: body mass index; EDSS: Expanded Disability Status Scale; SEM: standard error of mean.

| Table 1 |
|---|---|
| | RRMS (n = 12) | Healthy controls (n = 14) |
| Age (years) | 29.1 ± 1.3 (22–37) | 31.4 ± 1.1 (26–39) |
| Range | Mean ± SEM | Mean ± SEM |
| BMI (kg/m$^2$) | 20.38 ± 0.3 (18.17–23.88) | 21.2 ± 0.5 (18.91–23.32) |
| Range | Mean ± SEM | Mean ± SEM |
| EDSS | 3.7 ± 0.6 | – |
| Range | (1–6) | – |
| Initial symptoms$^a$ | Cranial nerve involvement; n (%) | 5 (42%) | – |
| | Motor weakness; n (%) | 4 (33%) | – |
| | Sensory symptoms; n (%) | 3 (25%) | – |
| Location of MS lesions$^b$ | Ventricular lesions; n (%) | 12 (100%) | – |
| | Juxtacortical lesions; n (%) | 9 (75%) | – |
| | Infratentorial lesions; n (%) | 7 (58%) | – |
| | Spinal cord lesions; n (%) | 0 (0%) | – |

BMI: body mass index; EDSS: Expanded Disability Status Scale; SEM: standard error of mean.

Values of age, BMI and EDSS are expressed as both mean ± SEM and range (min–max). Values of initial symptoms and location of MS lesions are expressed as number (percent). p-Value less than 0.05 were considered significant.

$^a$ Based on results of initial symptoms, all RRMS patients were monosymptomatic.

$^b$ Location of CNS lesions of MS patients were identified by MRI scan.
As shown in Fig. 1, plasma antioxidant levels (FRAP values) of the MS patients were significantly lower than the healthy subjects (p = 0.03).

3.1. The assessment of PBMCs viability by MTT assay

PBMCs were treated with the increasing concentration of melatonin (1 mM, 1 μM and 1 nM) for 12 h and cell viability was assessed by MTT assay. We observed no significant change in cell viability after exposure to 1 mM (97.8 ± 3.3%), 1 μM (94.5 ± 3.6%) and 1 nM (99.5 ± 3.6%) melatonin in comparison with untreated PBMCs (96.85 ± 4.4%) and the cell viability was more than 90% under treatment with these concentrations (Fig. 2).

Then, in order to find the optimum concentration of melatonin for further experiments, we performed a preliminary investigation, in which a range of melatonin concentration (1 mM, 1 μM and 1 nM) was tested for their effect on mRNA expression of all studied genes. Our results showed that melatonin concentration of 1 mM had the highest effect on mRNA expression of all studied genes (data not shown). Therefore, we selected treatment with melatonin at a concentration of 1 mM for further experiments.

3.2. Effect of melatonin on mRNA expression of SIRT1, MnSOD, and catalase

Melatonin treatment of PBMCs from RRMS patients resulted in a 2.6-fold increase in SIRT1 mRNA levels (p < 0.001) (Fig. 3a), in comparison with untreated cells. Moreover, an almost 2-fold increase in MnSOD (p = 0.003) (Fig. 3b) and catalase (p < 0.001) (Fig. 3c) mRNA levels was observed in the melatonin-treated cells from patients. In healthy subjects, melatonin caused a significant increase in mRNA expression of SIRT1 (2.4-fold change) and catalase (2.2-fold change), whereas melatonin treatment had no influence on the MnSOD mRNA level in PBMCs from healthy subjects (p = 0.001, p = 0.001, and p = 0.79, respectively) (Fig. 3a–c). The between-group analysis indicated that the difference between healthy subjects and patients for mRNA expression of SIRT1 in untreated cells was not statistically significant (p = 0.69), but the mRNA expression of catalase in untreated PBMCs from RRMS patients was significantly higher (p = 0.01) compared with that in healthy subjects. MS patients also showed a borderline significant decrease (p = 0.073) in MnSOD mRNA level in comparison with healthy subjects.

3.3. Effect of melatonin on the activity of SIRT1, MnSOD, and catalase

With regard to SIRT1 activity, an almost 1.5 fold increase was observed in both patients (p = 0.03) and the healthy group (p < 0.001) in the melatonin-treated cells (Fig. 4a). Melatonin treatment of PBMCs from healthy subjects had no effect on the activity of MnSOD compared with untreated cells (Fig. 4b). In contrast, the MnSOD activity in RRMS patients was significantly (p = 0.004) increased by 1.26-fold in the melatonin-treated cells compared with untreated cells (Fig. 4b). In addition, melatonin significantly increased catalase activity in PBMCs of both patients (p = 0.012) and healthy subjects (p = 0.011) by 1.47- and 1.72-fold, respectively (Fig. 4c). In the between group analysis, catalase activity in RRMS patients was significantly higher (p = 0.025) compared with healthy subjects, whereas MnSOD activity was significantly decreased in the patient group as compared with control subjects (p = 0.001). There was also a borderline significant decrease (p = 0.084) in SIRT1 activity in patients during relapse as compared with the healthy group.

3.4. The correlations of SIRT1 activity with MnSOD and catalase activities in MS patients and healthy subjects before and after melatonin treatment

Considering the important role of SIRT1, as a deacetylase enzyme in regulation of the activity of MnSOD and catalase (two central antioxidant enzymes) correlations of SIRT1 activity with MnSOD and catalase activities were evaluated in untreated PBMCs and melatonin-treated PBMCs of MS patients and healthy group (Table 2).

As depicted in this table, in healthy subjects, SIRT1 activity significantly correlated with catalase (r = 0.587, p = 0.027) and MnSOD activities (r = 0.669, p = 0.009) in untreated cells, while SIRT1 activity had no correlation with catalase activity (r = 0.045, p = 0.84) and MnSOD activity (r = 0.171, p = 0.594) in untreated cells from RRMS patients.

In PBMCs treated with melatonin, a significant correlation was observed between SIRT1 activity and catalase activity in both patients (r = 0.601, p = 0.039) and healthy subjects (r = 0.640, p = 0.014). There was no correlation between SIRT1 activity and MnSOD activity in melatonin-treated PBMCs of MS patients (r = 0.24, p = 0.453), while we found a significant correlation between SIRT1 activity and MnSOD activity in melatonin-treated PBMCs of healthy volunteers (r = 0.740, p = 0.002).

4. Discussion

There is strong evidence for the central role of oxidative stress in the initiation and progression of MS [5,38]. Recently, a limited number of studies have focused on the protective role of melatonin in the context of MS [30,32,39]; however, there are still many unanswered issues in this regard. As far as we know, this is the first study to demonstrate the effect of melatonin on gene expression and activity of antioxidant enzymes in PBMCs from MS patients and healthy subjects.

Consistent with studies showing defective antioxidant responses in MS patients in other tissues [5,40–42], we found a statistically significant decrease in the MnSOD activity in untreated PBMCs from MS
patients that was accompanied by a significant reduction in TAC in plasma. In addition, it was found that MnSOD mRNA expression and SIRT1 activity were decreased by 50% and 30%, respectively, in RRMS patients compared with healthy subjects, albeit with borderline significance. However, some studies have reported opposing results [11,43–44]. Furthermore, as expected, not all antioxidant enzymes were reduced in MS patients. To confirm this notion, mRNA expression and activity of catalase in untreated PBMCs from RRMS patients were significantly higher as compared with those in healthy subjects. Accordingly, it appears that antioxidant status is affected during the relapse phase in MS patients. However, the assessment of additional enzymatic and non-enzymatic antioxidants in PBMCs of MS patients will be required to further support this notion.

Superoxide dismutases and hydroperoxidases (catalase and GPx) are the first line of cell defenses against free radicals and reactive oxygen species (ROS) [44]. Accordingly, it is likely that our finding of increased activity of catalase in MS patients is interpreted as an attempt to overcome the oxidative stress milieu in these patients. In support of this concept, a recent study showed elevated catalase activity in response to microglial activation in an auto-regulatory manner in MS patients [45].

Although previous studies demonstrated the antioxidant effects of melatonin on other tissues and other pathological and physiological conditions [25,46]; we showed the favorable effects of melatonin on the defective antioxidant defense system in PBMCs of MS patients for the first time.

Here, we demonstrated that melatonin could reduce oxidative stress through a significant increase in activity and expression of MnSOD, catalase, and SIRT1 in PBMCs from MS patients (Fig. 5). In control subjects, melatonin significantly increased mRNA expression and activities of catalase and SIRT1. Since healthy subjects endure mild level of oxidative stress [47–48]; it can be useful to enhance antioxidant enzymes (Fig. 5).

With regard to patients, our results are partly in accordance with some clinical evidence regarding melatonin effect on other aspects of cellular defense against oxidative stress in MS, which was associated with an improvement in some aspects of quality of life [30–32]. However, in consistent with our results of increased MnSOD activity only in MS patients, Miller et al. showed that melatonin distinctly augmented the enzyme activity of SOD in the erythrocytes of MS patients with secondary progressive course. But, no alteration was observed in SOD activity in healthy subjects following melatonin supplementation [32].

To our knowledge, no mechanism has been reported regarding increased MnSOD activity only in RRMS patients upon melatonin treatment; however, some possibilities can be put forward to explain this finding. One plausible reason may be due to limited cellular capacity to confront oxidative stress as well as the limited ability of melatonin to regulate MnSOD enzyme activity. Another possible reason behind this finding could be related to the multi-faceted and complicated pathophysiology of MS disease. However, both molecular genetics and protein structural studies on MnSOD provide further mechanistic insights in this regard.

Given the apparent role of SIRT1 in the regulation of oxidative stress pathways [15,17–18] and dysregulation of oxidative stress pathways in SIRT1-knockout models [49–50] it raises the possibility that our results of a decrease in SIRT1 activity in untreated-PBMCs of MS patients, along with a decrease in TAC and also reduced levels of MnSOD gene expression and activity are responsible for the impaired antioxidant defense system in MS.

Animal model studies showed that SIRT1 activation ameliorates course of disease in EAE through preserving axonal function, improving neurological dysfunction and reducing neuronal loss-induced oxidative stress [51–52]. Notably, SIRT1 inhibition in MS patients
caused apoptosis in immune cells such as Jurkat cells, CD4+ and CD8+ [13]. Therefore, it is postulated that marked enhancement in SIRT1 activity after melatonin treatment reduces oxidative stress in MS patients and exerts beneficial effects in disease management.

Superoxide dimutases and catalase catalyze the dismutation of superoxide to H2O2 and H2O2 to water and oxygen, respectively. Oligodendrocytes as the myelin-forming cells of the central nervous system are highly susceptible to the deleterious effects of H2O2. However, if excess H2O2 are not effectively eliminated by antioxidant enzymes, it results in axonal damage and myelin degeneration [53]. Dysregulation of antioxidant defenses also causes the infiltration of leukocytes and these cells themselves produce inflammatory mediators, including ROS and various cytokines in a positive feedback loop [54]. Accordingly, targeting MnSOD and catalase may be beneficial in MS management.

In the present study, melatonin treatment of PBMCs from RRMS patients restored the decreased mRNA levels and MnSOD activity to almost basal levels of those seen in PBMCs from healthy subjects. Interestingly, melatonin could remarkably augment catalase activity and mRNA expression in MS patients, despite its higher activity and expression in MS patients compared with control subjects. This would suggest a countervailing and compensatory mechanism against oxidative challenge in MS. Similarly, melatonin exposure not only restored, but also bolstered decreased expression of complexes I–IV in mitochondria in cuprizone-induced demyelination model [55].

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Coefficients (r) and p-values are calculated by the Pearson correlation method. All untreated cells were treated with 0.1% of DMSO as a control.

Fig. 5. Effect of melatonin on SIRT1, MnSOD and catalase gene expressions and activities in PBMCs from MS patients and healthy subjects. In MS patients, melatonin (1 mM) could reduce oxidative stress in PBMCs through increase in mRNA expression and enzyme activity of SIRT1, MnSOD and catalase in PBMCs from MS patients (a). In healthy subjects, melatonin (1 mM) significantly increased mRNA expression and activity of catalase and SIRT1 (b). The vertical black arrow represents increase in enzyme activity compared with untreated cells. The vertical white arrow represents increase in mRNA expression compared with untreated cells. Fold changes in gene expression and enzyme activity following melatonin treatment were expressed as percentage (%) of untreated cells.
In the present study, SIRT1 activity was positively correlated with activities of MnSOD and catalase in PBMCs of healthy subjects both before and after melatonin treatment. While, it was shown a positive correlation between SIRT1 activity and catalase activity only in melatonin-treated PBMCs from RRMS patients. There is also evidence that SIRT1 and catalase interact as partners in other systems, including SIRT1, MnSOD and catalase in PBMCs. Hence, it appears that SIRT1 activity is essential for maintenance of T cell tolerance in mice. J. Clin. Invest. 119 (10) (Oct 2009) 3048–3058.


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Collectively, the present study partly addresses the protective effects of melatonin as a putative antioxidant, and its role in bolstering an inefficient antioxidant defense in PBMCs of MS patients. However, more studies in a large sample size are required to clarify the role of melatonin in MS management. Also, we would like to stress that our findings, along with others, can provide the basis for future studies, which may be able to comprehensively look at the role of antioxidant enzymes as therapeutic targets for MS treatment. However, the present study has some limitations. First, we could not thoroughly investigate the effect of melatonin on other genes involved in oxidative stress pathways. Moreover, from clinical point of view further studies are necessary to fully determine the effectiveness of melatonin treatment in MS.

5. Conclusion

Our findings suggest that antioxidant status is affected in PBMCs isolated from MS patients and melatonin could improve the impaired antioxidant defense through upregulating key antioxidant molecules including SIRT1, MnSOD and catalase in PBMCs. Hence, it appears that melatonin could exert favorable effects in MS patients. However, further studies evaluating the effect of melatonin on other enzymatic and non-enzymatic antioxidants in PBMCs are required to support this notion.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

Acknowledgments

We are thankful to the Tehran University of Medical Sciences for the financial support of this study (study number 91.130 17019).

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