

Research Article

Can Melatonin Act as an Antioxidant in Hydrogen Peroxide-Induced Oxidative Stress Model in Human Peripheral Blood Mononuclear Cells?

Soleleh Emamgholipour,¹ Arash Hossein-Nezhad,^{2,3} and Mohammad Ansari¹

¹Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, P.O. Box 14155-6447, Tehran, Iran

²Department of Medicine, Section of Endocrinology, Nutrition, and Diabetes, Vitamin D, Skin and Bone Research Laboratory, Boston University Medical Center, 85 E. Newton Street Fuller Building, Boston, MA 02118, USA

³Osteoporosis Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, P.O. Box 1411413137, Tehran, Iran

Correspondence should be addressed to Mohammad Ansari; ansarimo@sina.tums.ac.ir

Received 29 October 2015; Accepted 10 December 2015

Academic Editor: Phillip I. Bird

Copyright © 2016 Soleleh Emamgholipour et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Purpose. We aimed to investigate the possible effects of melatonin on gene expressions and activities of MnSOD and catalase under conditions of oxidative stress induced by hydrogen peroxide (H_2O_2) in peripheral blood mononuclear cells (PBMCs). **Materials and Methods.** PBMCs were isolated from healthy subjects and treated as follows: (1) control (only with 0.1% DMSO for 12 h); (2) melatonin (1 mM) for 12 h; (3) H_2O_2 (250 μ M) for 2 h; (4) H_2O_2 (250 μ M) for 2 h following 10 h pretreatment with melatonin (1 mM). The gene expression was evaluated by real-time PCR. MnSOD and catalase activities in PBMCs were determined by colorimetric assays. **Results.** Pretreatment of PBMCs with melatonin significantly augmented expression and activity of MnSOD which were diminished by H_2O_2 . Melatonin treatment of PBMCs caused a significant upregulation of catalase by almost 2-fold in comparison with untreated cells. However, activity and expression of catalase increased by 1.5-fold in PBMCs under H_2O_2 -induced oxidative stress compared with untreated cell. Moreover, pretreatment of PBMCs with melatonin resulted in a significant 1.8-fold increase in catalase expression compared to PBMCs treated only with H_2O_2 . **Conclusion.** It seems that melatonin could prevent from undesirable impacts of H_2O_2 -induced oxidative stress on MnSOD downregulation. Moreover, melatonin could promote inductive effect of H_2O_2 on catalase mRNA expression.

1. Introduction

Oxidative stress resulting from an imbalance between reactive oxygen species (ROS) production and neutralization plays an important role in the development of multiple pathophysiological states such as diabetes, atherosclerosis, cancers, neurodegenerative diseases, aging, and autoimmunity. In these conditions, antioxidant defense system is also impaired and subsequently increases oxidative burden [1–4].

Under aerobic situations, ROS are produced as a result of cellular metabolism (e.g., during electron transport in the mitochondria and in the endoplasmic reticulum), function of several oxidase enzymes (e.g., NADPH oxidases, xanthine

oxidase, and cytochrome p450 oxidase), and metal-catalyzed oxidative reactions. In normal situations, ROS participates in regulation of normal cellular functions. However, if excess ROS are not effectively eliminated by antioxidant defense system, it causes cellular damage by influencing gene expression and by oxidating DNA, proteins, and lipids. Antioxidant defense systems, both enzymatic and nonenzymatic, keep ROS in check and protect tissue from oxidative damage. Superoxide dismutases (SODs) including MnSOD, Cu/Zn-SOD, and extracellular SOD along with catalase are composed of the first line of defense against ROS. MnSOD is found in the mitochondrial matrix which dismutates superoxide to hydrogen peroxide (H_2O_2). Catalase is localized in

peroxisomes, mitochondria, nucleus, and sarcoplasm and converts H_2O_2 to water and oxygen [5–8]. Deficiencies in MnSOD and catalase are associated with development of a broad range of metabolic disorders, inflammatory conditions, autoimmunity, and cancers. There is also evidence that activation of antioxidant enzymes improves these conditions [9–11].

In the last decade, melatonin (N-acetyl-5-methoxy-tryptamine) has received a great deal of attention as a potential therapeutic strategy for the prevention of stress-related disorders. Melatonin is a pleiotropic hormone mainly secreted by pineal gland. Melatonin treatment has been also found to exert beneficial effects in metabolic disorders via regulating body weight, energy expenditure, and lipid and glucose metabolism [12]. This hormone also regulates circadian rhythms, inflammation pathways, and apoptosis. However, melatonin is known for its effects on oxidative stress pathways [8, 13]. It is evident that melatonin acts as an antioxidant through free radical scavenging properties as well as by its role in activating several antioxidant enzymes [14, 15].

Peripheral blood mononuclear cells (PBMCs), a heterogeneous population of immune cells, are helpful in revealing the complex nature of the disease, and in biomonitoring studies [16]. There is evidence that PBMCs are sensitive targets to oxidative stress and can reflect systemic symptoms of oxidative damage [17–19]. Despite evidence reporting the effects of melatonin on components of the cellular oxidative stress defense in various *in vivo* and *in vitro* studies, to our knowledge, no study has investigated the antioxidative effects of melatonin in oxidative stress model in PBMCs. In addition, there is no report on impact of H_2O_2 -induced oxidative stress on antioxidant enzymes in human PBMCs. In this study, we first investigated the impact of H_2O_2 -induced oxidative stress on antioxidant enzymes in PBMCs by studying the changes in MnSOD and catalase as two primary antioxidative defense enzymes. Secondly, we investigated the possible effect of melatonin against oxidative stress in human PBMCs.

2. Materials and Methods

2.1. Subjects. This experimental study was conducted on 14 healthy volunteers with an age between 20 and 40 years. Participants were excluded if they had any of the following: (1) history of malignancy, diabetes (types I and II), acute or chronic infection, and any known systemic disease; (2) medication with antioxidant supplements, anti-inflammatory drugs, and vitamins in the previous 6 months. It should be noted that none of the volunteers was currently smoking. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, and written informed consent was obtained from all subjects.

2.2. Isolation of PBMCs. After an overnight fast, peripheral blood sample (30 mL) was obtained from all volunteers and collected in sodium-heparin blood collection tubes. PBMC isolation was performed immediately using Ficoll-Hypaque (Lympholyte-H; Cedarlane Laboratories, Hornby,

ON, Canada) gradient centrifugation. Briefly, blood from each subject was diluted with equal volume of phosphate-buffered saline (PBS), placed on Ficoll medium and centrifuged at $600 \times g$ for 30 min at room temperature. The PBMC layer was collected and washed twice with sterile PBS solution (GIBCO; Invitrogen Laboratories, UK) by centrifugation at $200 \times g$ for 15 min. The cell viability was assessed by trypan blue exclusion assay.

2.3. Cell Culture and Treatment. Freshly isolated PBMCs were suspended in 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). PBMCs were then plated at a density of 2×10^6 cells/well and 4×10^6 cells/well in 12- and 6-well flat-bottom plates, respectively.

After overnight incubation at $37^\circ C$ with 5% CO_2 , PBMCs were treated as follows:

- (1) Control group: PBMCs incubated only with 0.1% DMSO for 12 h.
- (2) Melatonin group: PBMCs treated with 1 mM melatonin for 12 h.
- (3) H_2O_2 group: PBMCs treated with $250 \mu M H_2O_2$ for 2 h.
- (4) Melatonin + H_2O_2 group: PBMCs treated with $250 \mu M H_2O_2$ for 2 h following 10 h pretreatment with 1 mM melatonin.

Finally, the cells were collected and stored at $-80^\circ C$ for further experiments. It should be mentioned that melatonin was dissolved in DMSO and diluted in RPMI 1640 before use. The final concentration of DMSO in the culture medium was 0.1%.

2.4. Evaluation of Cell Viability under Melatonin Treatment. The cell viability was performed 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 25×10^4 /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 $37^\circ C$ in the humidified atmosphere with 5% CO_2 . Then, PBMCs were washed three times with phosphate buffer saline and the MTT labeling reagent (500 $\mu g/mL$) was added to wells and PBMCs were further incubated for 4 h at $37^\circ C$. The media were discarded and the blue formazan crystals were solubilized with DMSO. The percentage of viable cells was calculated by the following formula: a ratio of absorbance of treated cells to control cells (treated with 0.1% DMSO) at 540 nm.

The MTT assay was also performed to evaluate cell viability in the presence of different concentration of H_2O_2 (50 μM , 100 μM , 250 μM , and 500 μM) with the same procedure mentioned above.

2.5. Quantitative Real-Time PCR. RNA extraction from PBMCs was performed using a Total RNA Extraction Mini-prep kit (Viogene, Taiwan) based on the manufacturer's instruction. The RNA purity and concentration were determined using a NanoDrop spectrophotometer. The RNA quality was checked by agarose gel electrophoresis.

The complementary DNA (cDNA) synthesis was performed on one microgram of DNase-treated RNA using Revert Aid First Strand cDNA Synthesis kit (Thermo Scientific, Fermentas, USA).

Real-time PCR was carried out in a Rotor Gene real-time thermocycler (Qiagen, Hilden, Germany) using SYBR Green detection kit (Takara Bio, Otsu, Japan) according to the manufacturer's protocol. Primers for target genes (MnSOD and catalase) and housekeeping gene, β -actin, were purchased from Qiagen (Hilden, Germany). Standard curves were generated for all studied genes to evaluate the linear range of the real-time PCR before performing the assay on test samples. The correlation coefficients of all the standard curves were more than 0.95 indicating a good linearity, and all test samples were verified to be within this range. It should be noted that melting curve analysis and subsequent gel electrophoresis were used to confirm the specificity of PCR products. Relative gene expression was normalized to β -actin and calculated as $2^{-\Delta CT}$ using the formula: $2^{-(Ct \text{ target gene} - Ct \beta\text{-actin})}$.

2.6. The Measurement of MnSOD Activity. MnSOD activity was measured in PBMCs using commercially available kit (SOD activity Enzo Life Sciences, USA). The assay is based on the principle that MnSOD neutralizes superoxide anions produced by the xanthine/xanthine oxidase system and subsequently inhibits the reduction of WST-1 (water soluble tetrazolium salt) to WST-1 formazan. Briefly, PBMCs were harvested, washed with ice-cold 1x PBS, and lysed as described in kit protocol. Preincubation of the cell lysate with 2 mM potassium cyanide (KCN) was performed to inactivate both Cu/Zn-SOD and extracellular SOD.

It is important to note that the linear range of the assay was evaluated by generating a standard curve prior to measuring the MnSOD activity. Specifically, we created a serial dilution of the cell extracts, ranging from 0.5 μ g/25 μ L to 50 μ g/25 μ L, with 1x SOD buffer. Afterward, the percentage of inhibition of the formation rate of WST-1 formazan was compared with a standard curve generated with serial dilutions of the SOD standard. The SOD standard, WST-1 reagent, and 1x SOD buffer were supplied in kit form. Protein concentration was determined using Bradford method [20]. MnSOD activity values were expressed as units/mg of protein.

2.7. The Measurement of Catalase Activity. Catalase activity in PBMCs (2×10^6 cells/well) was determined using the catalase assay kit (Ab83464; Abcam, Cambridge, MA, USA) as per the manufacturer's instructions. In this assay, catalase decomposes H_2O_2 to water and oxygen, and then unconverted H_2O_2 reacts with OxiRed probe to produce a product that can be measured 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 expressed as units/mg of protein.

2.8. Statistical Analysis. All data were analyzed using SPSS 19 (SPSS Inc., Chicago, IL, USA) and presented as mean \pm standard error of the mean (SEM). Differences between the groups were done using one way ANOVA followed by the *post hoc* Tukey test to compare all pairs of groups. Comparative CT method [21] was used for analysis of the gene expression. Statistical significance was considered at a *p* value < 0.05 , unless noted.

3. Results

3.1. Effect of Melatonin on Cell Viability in PBMC Cultures. PBMCs were treated with the increasing concentration of melatonin (1 mM, 1 μ M, 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 ($98.9 \pm 4.1\%$), 1 μ M ($94.6 \pm 2.8\%$), and 1 nM ($97.7 \pm 4.5\%$) melatonin in comparison with untreated PBMCs and the cell viability was more than 90% under treatment with these concentrations (Figure 1).

To find the optimum concentration of melatonin for further experiments, we performed a preliminary study, in which melatonin concentrations of 1 mM, 1 μ M, and 1 nM were evaluated for their effect on MnSOD and catalase mRNA expression. Our results showed that melatonin concentration of 1 mM had the highest effect on mRNA expression (data not shown). Accordingly, we selected treatment with melatonin at a concentration of 1 mM for next experiments.

3.2. Effect of H_2O_2 on Cell Viability in PBMCs Cultures. To evaluate the influence of H_2O_2 treatment on cell viability, PBMCs were treated with increasing concentration of H_2O_2 (50 μ M, 100 μ M, 250 μ M, and 500 μ M) for 2 h and MTT assay was performed. The treatment of PBMCs with H_2O_2 at concentration of 50 μ M, 100 μ M, and 250 μ M showed no cytotoxic effect on PBMCs and cell viability was more than 90% under PBMCs treatment with these concentrations. However, the exposure of PBMCs with H_2O_2 at 500 μ M for 2 h significantly diminished the cell viability to 87% (*p* = 0.02) (Figure 2).

To induce oxidative stress for further experiments, H_2O_2 at concentration of 250 μ M was selected as we found no significant decrease in cell viability in this concentration. Additionally, based on preliminary data, alteration of MnSOD and catalase gene expression was the most pronounced at this concentration compared with 50 μ M and 100 μ M of H_2O_2 .

3.3. The Assessment of mRNA Expression and Activity of MnSOD under Treatment of PBMCs with Melatonin, H_2O_2 , and Melatonin plus H_2O_2 . We found no alteration in mRNA expression and enzyme activity of MnSOD in PBMCs treated with 1 mM melatonin compared to untreated cells (Figures 3(a) and 3(b)).

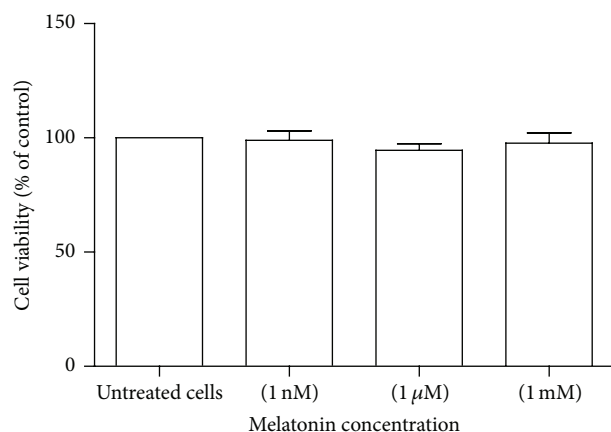


FIGURE 1: Assessment of PBMCs viability in response to melatonin treatment. Cells were incubated with increasing concentrations of melatonin (1 nM, 1 μM, and 1 mM) for 12 h and cell viability was determined by MTT assay. The MTT assay was performed in triplicate and data are reported from the mean of triplicates. Data are expressed as means ± SEM.

To address the possible effect of oxidative stress on expression and enzyme activity of antioxidant enzymes, PBMCs were treated with 250 μM H₂O₂ for 2 h. Treatment of PBMCs with 250 μM H₂O₂ resulted in a 2.5-fold decrease in MnSOD mRNA levels (Figure 3(a)), in comparison with untreated cell, although it was not statistically significant ($p = 0.19$) (Figure 3(a)). Moreover, H₂O₂-induced oxidative stress significantly diminished MnSOD enzyme activity in PBMCs to 25% of baseline level ($p = 0.04$) (Figure 3(b)).

Interestingly, pretreatment of PBMCs with melatonin (1 mM) prior to exposure to H₂O₂ (250 μM) caused a significant 2.2-fold increase in MnSOD mRNA levels in comparison with PBMCs treated only with H₂O₂ ($p < 0.001$) (Figure 3(a)). Pretreatment of PBMCs with melatonin before addition of H₂O₂ also significantly suppressed the decreasing effect of H₂O₂ on MnSOD enzyme activity by 30% ($p = 0.005$) (Figure 3(b)).

3.4. The Assessment of mRNA Expression and Activity of Catalase under Treatment of PBMCs with Melatonin, H₂O₂, and Melatonin plus H₂O₂. Melatonin treatment of PBMCs resulted in an almost 2.2-fold increase in catalase mRNA expression ($p = 0.03$) (Figure 4(a)), in comparison with untreated cells. With regard to catalase activity, an approximately 80% increase was found in PBMCs treated with melatonin (1 mM) in comparison with untreated cells ($p = 0.015$) (Figure 4(b)).

Catalase mRNA expression increased by 1.5-fold in PBMCs under H₂O₂-induced oxidative stress compared with untreated cell although it was not statistically significant ($p = 0.6$). Moreover, H₂O₂-induced oxidative stress significantly ($p = 0.044$) elevated catalase enzyme activity in PBMCs compared with untreated cell. Melatonin treatment (1 mM) prior to PBMCs exposure to H₂O₂ (250 μM) caused a significant 1.8-fold increase in catalase mRNA levels in comparison with

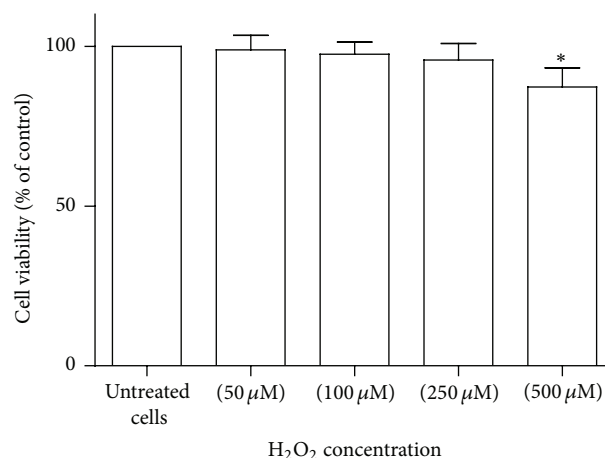


FIGURE 2: Assessment of PBMCs viability in response to H₂O₂ treatment. Cells were incubated with increasing concentrations of H₂O₂ (50 μM, 100 μM, 250 μM, and 500 μM) for 2 h and cell viability was determined by MTT assay. The MTT assay was performed in triplicate and data are reported from the mean of triplicates. Data are expressed as means ± SEM. * $p < 0.05$.

PBMCs treated only with H₂O₂ ($p < 0.001$) (Figure 3(a)). Moreover, melatonin treatment of PBMCs before addition of H₂O₂ nonsignificantly ($p = 0.5$) enhanced the inductive effect of oxidative stress on catalase enzyme activity (Figure 4(b)).

4. Discussion

The role of melatonin in regulating antioxidant defense system and study on its protective effects against oxidative insult have been identified in various *in vivo* and *in vitro* studies [8, 15]. There is evidence that PBMCs are helpful tools to study the status of antioxidant defense system [17–19]. In this study, we examined, for the first time, the melatonin effect against oxidative stress in human PBMCs. In our model system, PBMCs were treated with H₂O₂ as a mediator of oxidative stress.

It is well established that high amount of oxidative stress overwhelms antioxidant defense machinery resulting in oxidative injury [22, 23]. In line with this notion, we found a marked decrease in mRNA expression and enzyme activity of MnSOD in H₂O₂ treated PBMCs as compared with untreated cells, suggesting the deleterious impacts of oxidative stress on the antioxidant system in PBMC.

However, the catalase mRNA expression and activity were increased following PBMCs exposure to oxidative stress. This observation reflects an adaptive response and a compensatory mechanism for the PBMCs against oxidative stress. This observation corroborates several lines of evidence indicating that cells respond to oxidant challenge with selective induction of certain antioxidant enzymes. In line with this notion, exposure to H₂O₂ caused an increase in catalase levels and activity in rat astroglial cells, in human retinal pigment epithelial cells, in hamster tracheal epithelial cells,

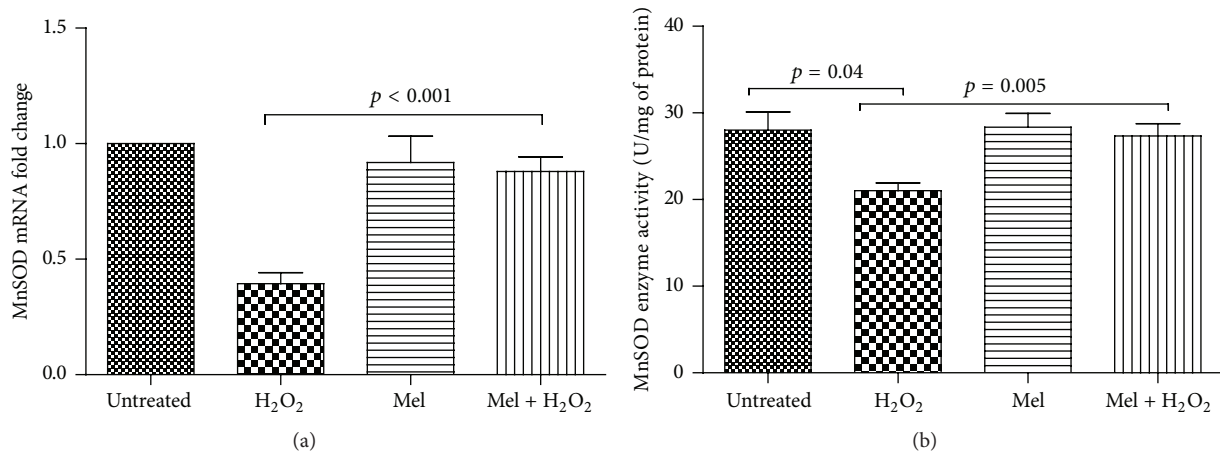


FIGURE 3: Effect of melatonin, H₂O₂, and melatonin plus H₂O₂ on (a) mRNA expression and (b) enzyme activity of MnSOD in PBMCs from healthy subjects. PBMCs were treated as follows: untreated (only with 0.1% DMSO for 12 h); melatonin (1 mM) for 12 h; H₂O₂ (250 μM) for 2 h; and melatonin plus H₂O₂ group receiving H₂O₂ (250 μM) for 2 h following 10 h pretreatment with melatonin (1 mM). All untreated cells were treated with 0.1% of DMSO as a control. Data are expressed as mean ± SEM.

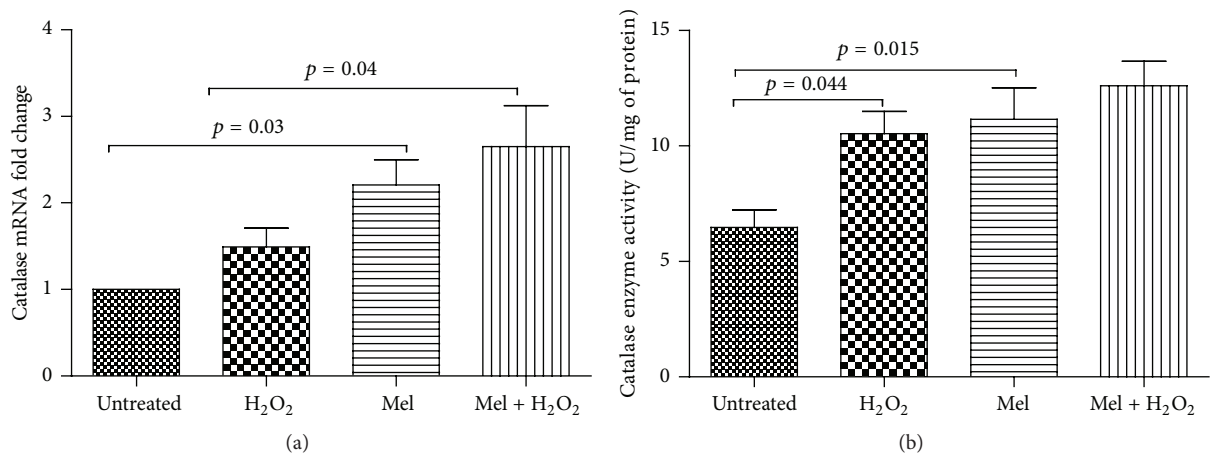


FIGURE 4: Effect of melatonin, H₂O₂, and melatonin plus H₂O₂ on (a) mRNA expression and (b) enzyme activity of catalase in PBMCs from healthy subjects. PBMCs were treated as follows: untreated (only with 0.1% DMSO for 12 h); melatonin (1 mM) for 12 h; H₂O₂ (250 μM) for 2 h; and melatonin plus H₂O₂ group receiving H₂O₂ (250 μM) for 2 h following 10 h pretreatment with melatonin (1 mM). All untreated cells were treated with 0.1% of DMSO as a control. Data are expressed as mean ± SEM.

and in hepatoma cells [24–26]. Some studies also showed that oxidative stress downregulated MnSOD in several oxidative stress models induced by H₂O₂ and other oxidants [27, 28]. However, other studies have reported differential regulation of antioxidant enzymes in exposure to oxidative stress [24, 29].

Under increased oxidative stress conditions, peroxynitrite, as a potent reactive oxidant, is produced by reaction between nitric oxide and superoxide anion. It was found that peroxynitrite severely diminishes MnSOD activity by tyrosine nitration both *in vitro* and *in vivo* [30]. Additionally, treatment of rat PBMCs with 100 μM H₂O₂ for 1 h caused an accumulation of intracellular superoxide radicals [17]. It is also evident that oxidative addition of superoxide anions

to Mn(II)SOD inhibits MnSOD activity through conformational change [31]. Accordingly, the reduced activity of MnSOD under oxidative stress conditions may be related to posttranslational modification of MnSOD and likely its impaired transcriptional regulation. However, further studies are required to identify the responsible mechanism(s).

As summarized above, there is still no general consensus among literature about pattern of antioxidant enzymes in response to oxidative stress. It may be related to the type of cellular model used, the type and concentration of oxidant agent, and incubation time.

Oxidative stress-induced downregulation of MnSOD activity causes superoxide radical accumulation and superoxide can be converted to hydroxyl radicals in the presence

of transition metals (e.g., Fenton reaction). Hydroxyl radical is the most damaging ROS produced by cells [6, 32, 33]. Hence, targeting MnSOD in conjugation with catalase as a well-known H₂O₂ removing enzyme is a potential approach for the treatment of oxidative stress-related conditions.

Interestingly, in our study, melatonin significantly reversed H₂O₂-induced downregulation of MnSOD to normal levels in PBMCs. Moreover, melatonin pretreatment promotes the inductive effect of oxidative stress on catalase expression. It would permit a countervailing and compensatory mechanism against oxidative challenge.

It was shown that treatment of 3T3-IL preadipocytes with melatonin (1 mM) increased activity of catalase and MnSOD [34]; however, Mayo et al. found no change in mRNA levels for antioxidant enzymes, Cu/Zn-SOD, MnSOD, and glutathione peroxidase in nondifferentiated PC12 cells and the human neuroblastoma cells after treatment with 0.1 mM melatonin [35]. Furthermore, based on a more recent study, melatonin alleviated impaired antioxidant enzyme activity in erythrocytes of multiple sclerosis patients [36].

There is evidence that melatonin administration preserved antioxidant status and SOD to normal levels in alloxan-induced oxidative insult in rats [37]. Our findings also corroborate the study of Dzięgiel et al. in which melatonin induced activity of SOD and catalase in myocardial cells of rats in the course of oxidative stress induced by doxorubicin. They found that activities of glutathione peroxidase, SOD, and catalase were higher in rats receiving doxorubicin and/or doxorubicin plus melatonin than in controls. Treatment of rats with doxorubicin plus melatonin markedly increased catalase activity in comparison with rats intoxicated with doxorubicin alone [38].

Although the exact mechanism in which melatonin regulates antioxidant enzymes has not been recognized, several possibilities derived from experimental studies should be considered. Although pharmacological concentrations of melatonin (more than nanomolar concentration) stimulate gene expression and activity of antioxidant enzymes, these quantities of melatonin may act as a direct radical scavenger. This, in turn, alters the cellular redox state and subsequently regulates antioxidant enzyme activity and/or gene expression [15, 39].

It is well established that melatonin exerts many of its roles by its interaction with membrane receptors (MT1 and MT2) and with RZR/ROR receptors which are located in the nucleus. The existence of melatonin receptors is reported in immune cell including lymphocytes, monocytes, regulatory cells, and peripheral blood nuclear cells in humans. It has been proposed that melatonin regulates antioxidant enzyme activity via interacting with calmodulin, which in turn inactivates RORα transcriptional activity and increments antioxidant enzyme activity and expression. Melatonin also restrains RORα constitutive activity through MT1 receptor [40, 41].

It is proposed that melatonin suppresses the degradation of the nuclear factor-E2-related factor (Nrf2) and increases its nuclear translocation via its interacting with ubiquitin ligase. In the nucleus, Nrf2 stimulates the transcription of

antioxidant enzymes (e.g., MnSOD and catalase) through binding to the antioxidant response element (ARE) [42]. However further experimental studies are needed to unravel the related molecular mechanism.

Accordingly, the present study can partly open a new window into the protective effect of melatonin on impaired oxidative stress defense in PBMCs under oxidative stress challenge. Additionally, due to the cross talk between inflammation and oxidative stress [43], the antioxidant role of melatonin under elevated oxidative stress conditions can indirectly counteract the undesirable impacts of ROS on dysregulation of inflammatory pathways. However, some limitation of this study merits consideration here as we could not thoroughly investigate the effect of melatonin on other antioxidant enzymes.

5. Conclusion

It appears that melatonin treatment could alleviate the impaired antioxidant defense system in human PBMCs as indicated by a pronounced upregulation of MnSOD and catalase. However, more investigations are necessary to fully determine the effectiveness of melatonin treatment on oxidative stress-related disorders.

Conflict of Interests

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

Acknowledgment

The authors are thankful to the Tehran University of Medical Sciences for financial support of this study (study no. 91.1.30 17019).

References

- [1] M. T. Lin and M. F. Beal, "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," *Nature*, vol. 443, no. 7113, pp. 787–795, 2006.
- [2] B. C. Dickinson and C. J. Chang, "Chemistry and biology of reactive oxygen species in signaling or stress responses," *Nature Chemical Biology*, vol. 7, no. 8, pp. 504–511, 2011.
- [3] T. Finkel, "Oxidant signals and oxidative stress," *Current Opinion in Cell Biology*, vol. 15, no. 2, pp. 247–254, 2003.
- [4] E. Ortona, P. Margutti, P. Matarrese, F. Franconi, and W. Malorni, "Redox state, cell death and autoimmune diseases: a gender perspective," *Autoimmunity Reviews*, vol. 7, no. 7, pp. 579–584, 2008.
- [5] V. Adler, Z. Yin, K. D. Tew, and Z. Ronai, "Role of redox potential and reactive oxygen species in stress signaling," *Oncogene*, vol. 18, no. 45, pp. 6104–6111, 1999.
- [6] J. T. Hancock, R. Desikan, and S. J. Neill, "Role of reactive oxygen species in cell signalling pathways," *Biochemical Society Transactions*, vol. 29, no. 2, pp. 345–350, 2001.
- [7] W. Dröge, "Free radicals in the physiological control of cell function," *Physiological Reviews*, vol. 82, no. 1, pp. 47–95, 2002.

- [8] C. Tomas-Zapico and A. Coto-Montes, "Melatonin as antioxidant under pathological processes," *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*, vol. 1, no. 1, pp. 63–82, 2007.
- [9] J. M. Matés and F. Sánchez-Jiménez, "Antioxidant enzymes and their implications in pathophysiological processes," *Frontiers in Bioscience*, vol. 4, pp. D339–D345, 1999.
- [10] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease," *International Journal of Biochemistry and Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.
- [11] J. M. Matés, C. Pérez-Gómez, and I. N. De Castro, "Antioxidant enzymes and human diseases," *Clinical Biochemistry*, vol. 32, no. 8, pp. 595–603, 1999.
- [12] M. Navarro-Alarcón, F. J. Ruiz-Ojeda, R. M. Blanca-Herrera et al., "Melatonin and metabolic regulation: a review," *Food and Function*, vol. 5, no. 11, pp. 2806–2832, 2014.
- [13] A. Carpentieri, G. Díaz de Barboza, V. Areco, M. Peralta López, and N. Tolosa De Talamoni, "New perspectives in melatonin uses," *Pharmacological Research*, vol. 65, no. 4, pp. 437–444, 2012.
- [14] A. Galano, D. X. Tan, and R. J. Reiter, "Melatonin as a natural ally against oxidative stress: a physicochemical examination," *Journal of Pineal Research*, vol. 51, no. 1, pp. 1–16, 2011.
- [15] C. Rodriguez, J. C. Mayo, R. M. Sainz et al., "Regulation of antioxidant enzymes: a significant role for melatonin," *Journal of Pineal Research*, vol. 36, no. 1, pp. 1–9, 2004.
- [16] V. D. F. de Mello, M. Kolehmanien, U. Schwab, L. Pulkkinen, and M. Uusitupa, "Gene expression of peripheral blood mononuclear cells as a tool in dietary intervention studies: what do we know so far?" *Molecular Nutrition & Food Research*, vol. 56, no. 7, pp. 1160–1172, 2012.
- [17] H.-Y. Chiu, L.-Y. Tsao, and R.-C. Yang, "Heat-shock response protects peripheral blood mononuclear cells (PBMCs) from hydrogen peroxide-induced mitochondrial disturbance," *Cell Stress and Chaperones*, vol. 14, no. 2, pp. 207–217, 2009.
- [18] N. Cases, A. Sureda, I. Maestre et al., "Response of antioxidant defences to oxidative stress induced by prolonged exercise: antioxidant enzyme gene expression in lymphocytes," *European Journal of Applied Physiology*, vol. 98, no. 3, pp. 263–269, 2006.
- [19] P. Tauler, A. Aguiló, I. Gimeno, E. Fuentespina, J. A. Tur, and A. Pons, "Response of blood cell antioxidant enzyme defences to antioxidant diet supplementation and to intense exercise," *European Journal of Nutrition*, vol. 45, no. 4, pp. 187–195, 2006.
- [20] N. J. Kruger, "The Bradford method for protein quantitation," in *The Protein Protocols Handbook*, Springer Protocols Handbooks, pp. 17–24, Humana Press, Totowa, NJ, USA, 2009.
- [21] T. D. Schmittgen and K. J. Livak, "Analyzing real-time PCR data by the comparative CT method," *Nature Protocols*, vol. 3, no. 6, pp. 1101–1108, 2008.
- [22] N. Ercal, H. Gurer-Orhan, and N. Aykin-Burns, "Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage," *Current Topics in Medicinal Chemistry*, vol. 1, no. 6, pp. 529–539, 2001.
- [23] M. Frisard and E. Ravussin, "Energy metabolism and oxidative stress," *Endocrine*, vol. 29, no. 1, pp. 27–32, 2006.
- [24] E. Röhrdanz and R. Kahl, "Alterations of antioxidant enzyme expression in response to hydrogen peroxide," *Free Radical Biology and Medicine*, vol. 24, no. 1, pp. 27–38, 1998.
- [25] S. Shull, N. H. Heintz, M. Periasamy et al., "Differential regulation of antioxidant enzymes in response to oxidants," *Journal of Biological Chemistry*, vol. 266, no. 36, pp. 24398–24403, 1991.
- [26] D. J. Tate Jr., M. V. Miceli, and D. A. Newsome, "Phagocytosis and H₂O₂ induce catalase and metallothionein gene expression in human retinal pigment epithelial cells," *Investigative Ophthalmology and Visual Science*, vol. 36, no. 7, pp. 1271–1279, 1995.
- [27] I. K. Lee, K. A. Kang, R. Zhang, B. J. Kim, S. S. Kang, and J. W. Hyun, "Mitochondria protection of baicalein against oxidative damage via induction of manganese superoxide dismutase," *Environmental Toxicology and Pharmacology*, vol. 31, no. 1, pp. 233–241, 2011.
- [28] L. A. MacMillan-Crow, J. P. Crow, and J. A. Thompson, "Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues," *Biochemistry*, vol. 37, no. 6, pp. 1613–1622, 1998.
- [29] E. Röhrdanz, G. Schmuck, S. Ohler, Q.-H. Tran-Thi, and R. Kahl, "Changes in antioxidant enzyme expression in response to hydrogen peroxide in rat astroglial cells," *Archives of Toxicology*, vol. 75, no. 3, pp. 150–158, 2001.
- [30] D. Candas and J. J. Li, "MnSOD in oxidative stress response-potential regulation via mitochondrial protein influx," *Antioxidants and Redox Signaling*, vol. 20, no. 10, pp. 1599–1617, 2014.
- [31] A. S. Hearn, M. E. Stroupe, D. E. Cabelli et al., "Kinetic analysis of product inhibition in human manganese superoxide dismutase," *Biochemistry*, vol. 40, no. 40, pp. 12051–12058, 2001.
- [32] J. K. Andersen, "Oxidative stress in neurodegeneration: cause or consequence?" *Nature Medicine*, vol. 10, supplement, pp. S18–S25, 2004.
- [33] Y. Gilgun-Sherki, E. Melamed, and D. Offen, "The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy," *Journal of Neurology*, vol. 251, no. 3, pp. 261–268, 2004.
- [34] M. Adamczyk-Sowa, P. Sowa, K. Zwirska-Korczala, K. Pierzchala, G. Bartosz, and I. Sadowska-Bartosz, "Role of melatonin receptor MT₂ and quinone reductase ii in the regulation of the redox status of 3T3-L1 preadipocytes in vitro," *Cell Biology International*, vol. 37, no. 8, pp. 835–842, 2013.
- [35] J. C. Mayo, R. M. Sainz, I. Antolín, F. Herrera, V. Martin, and C. Rodriguez, "Melatonin regulation of antioxidant enzyme gene expression," *Cellular and Molecular Life Sciences*, vol. 59, no. 10, pp. 1706–1713, 2002.
- [36] E. Miller, A. Walczak, I. Majsterek, and J. Kedziora, "Melatonin reduces oxidative stress in the erythrocytes of multiple sclerosis patients with secondary progressive clinical course," *Journal of Neuroimmunology*, vol. 257, no. 1–2, pp. 97–101, 2013.
- [37] M. M. Sailaja Devi, Y. Suresh, and U. N. Das, "Preservation of the antioxidant status in chemically-induced diabetes mellitus by melatonin," *Journal of Pineal Research*, vol. 29, no. 2, pp. 108–115, 2000.
- [38] P. Dzięgiel, E. Murawska-Ciałowicz, Z. Jethon et al., "Melatonin stimulates the activity of protective antioxidative enzymes in myocardial cells of rats in the course of doxorubicin intoxication," *Journal of Pineal Research*, vol. 35, no. 3, pp. 183–187, 2003.
- [39] D. S. Maharaj, B. D. Glass, and S. Daya, "Melatonin: new places in therapy," *Bioscience Reports*, vol. 27, no. 6, pp. 299–320, 2007.
- [40] S. R. Pandi-Perumal, I. Trakht, V. Srinivasan et al., "Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways," *Progress in Neurobiology*, vol. 85, no. 3, pp. 335–353, 2008.

- [41] C. Tomás-Zapico and A. Coto-Montes, "A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes," *Journal of Pineal Research*, vol. 39, no. 2, pp. 99–104, 2005.
- [42] J. Vriend and R. J. Reiter, "The Keap1-Nrf2-antioxidant response element pathway: a review of its regulation by melatonin and the proteasome," *Molecular and Cellular Endocrinology*, vol. 401, pp. 213–220, 2015.
- [43] A. Sánchez, A. C. Calpena, and B. Clares, "Evaluating the oxidative stress in inflammation: role of melatonin," *International Journal of Molecular Sciences*, vol. 16, no. 8, pp. 16981–17004, 2015.