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Molecular, Biological, Physiological and Clinical Aspects of Melatonin

Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome

Abstract: Experimental studies have proven that melatonin has many beneficial pleiotropic actions. The aim of this study was to assess melatonin efficacy in patients with metabolic syndrome (MS). The study included 33 healthy volunteers (who were not treated with melatonin) and 30 patients with MS, who did not respond to 3-month lifestyle modification. Patients with MS were treated with melatonin (5 mg/day, 2 hr before bedtime) for 2 months. The following parameters were studied: systolic and diastolic blood pressure (SBP, DBP), levels of glucose, serum lipids, C-reactive protein, fibrinogen, activities of antioxidative enzymes: catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), thiobarbituric acid reactive substrates (TBARS). After 2-month therapy in comparison with baseline, the following significant changes were measured: systolic blood pressure (132.8 ± 9.8 versus 120.5 ± 11.0 mmHg, $P < 0.001$), DBP (81.7 ± 8.8 versus 75 ± 7.4 mmHg, $P < 0.01$), low-density lipoprotein cholesterol (LDL-C) (149.7 ± 26.4 versus 139.9 ± 30.2 mg/dL, $P < 0.05$), TBARS (0.5 ± 0.2 versus 0.4 ± 0.1 μM/gHb, $P < 0.01$), and CAT (245.9 ± 46.9 versus 276.8 ± 39.4 U/gHb). Melatonin administered for 2 months significantly improved antioxidative defense (increase in CAT activity, decrease in TBARS level) and lipid profile (decrease in LDL-C), and lowered blood pressure. We conclude that melatonin therapy may be of benefit for patients with MS, particularly with arterial hypertension. Further studies with higher doses of melatonin or prolonged supplementation are awaited.

Marzena Koziróg[1], Adam Rafał Poliwczak[1], Piotr Duchnowicz[2], Maria Koter-Michalak[2], Joanna Sikora[3] and Marlena Broncel[1]

[1]Department of Internal Diseases and Clinical Pharmacology, Medical University of Lodz; [2]Department of Environmental Pollution Biophysics, University of Lodz; [3]Department of Pharmaceutical Chemistry and Drug Analysis, Medical University of Lodz, Lodz, Poland

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Address reprint requests to Marlena Broncel, Department of Internal Diseases and Clinical Pharmacology, Medical University of Lodz, Kniaziewicza Street 1/5, 91-347 Lodz, Poland. E-mail: marlena.broncel@umed.lodz.pl

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Introduction

The prevalence of metabolic syndrome (MS) has increased in recent decades. According to recent guidelines, we recognize MS when at least three of the five following criteria are present: visceral obesity, hypertriglyceridemia, decreased HDL cholesterol level, elevated blood pressure, and elevated fasting glucose [1]. Although visceral obesity is not necessary to recognize MS, it seems that the obesity epidemic is associated with multiplication in society of metabolic risk factors for cardiovascular diseases. It is estimated that 50–65% of the European population (200 million people) is overweight or obese. MS is associated with a number of pathological processes: elevated oxidative stress, activation of inflammatory cytokines, and prothrombotic mediators. A prolonged state of oxidative stress results in reduction in the antioxidative enzyme activities and excessive peroxidation of lipids. Impaired antioxidative–prooxidative balance may contribute to atherogenesis. Patients with MS are at a three times greater risk of acute coronary syndrome, two times greater risk of death, and five times greater risk of developing type 2 diabetes [2]. Results of MS treatment that include lifestyle modification and pharmacotherapy of MS components are not satisfactory. Thus, any drug that could be of benefit in MS subjects is keenly awaited.

Melatonin is an important component of the circadian system. There is, however, an increasing set of data documenting that melatonin is a potent antioxidant [3–5]. Numerous experimental studies have demonstrated that melatonin increases activities of antioxidative enzymes and reduces oxidative damage [6–8]. There is also increasing evidence that melatonin lowers oxidative stress in humans [9–11]. Moreover, numerous studies have shown that melatonin lowers blood pressure (BP), which is also of benefit to patients with MS [12–15]. Experimental findings also suggest that melatonin reduces inflammatory processes [16]. Collectively, these data suggest that melatonin may be useful in patients with MS, which is recognized as a chronic oxidative stress state. The aim of this study was to evaluate the influence of exogenous melatonin on antioxidative enzymes, lipid peroxidation, serum lipids, fasting glucose, C-reactive protein (CRP), fibrinogen, and blood pressure in patients with MS.

Material and methods

Subjects

The study group included 30 patients with MS who did not respond to 3-month lifestyle modification. The control group included 33 healthy volunteers matched for age and sex. Patients were eligible for the study if they met the following criteria: (i) waist circumference (≥80 cm for women and ≥94 cm for men), (ii) triglyceride (TG) level > 150 mg/dL (1.7 mM), (iii) high-density lipoprotein cholesterol (HDL-C) level < 40 mg/dL (1.0 mM) for men and < 50 mg/dL (1.3 mM) for women, and (iv) systolic blood pressure 160 mmHg ≥ (SBP) > 130 mmHg and/or diastolic blood pressure 100 mmHg ≥ (DBP) > 85 mmHg. The exclusion criteria were as follows: (i) secondary dyslipidemia in the course of autoimmune disorders, thyroid diseases, chronic pancreatitis, nephritic syndrome, liver, and biliary tract disease or alcoholism, (ii) any acute or chronic inflammatory processes, (iii) congestive heart failure, (iv) coronary artery disease, (v) history of myocardial infarction, stroke, or intermittent claudication, (vi) moderate or severe arterial hypertension (WHO/ISH grade 2 or 3), (vii) diabetes mellitus, (viii) impaired renal or hepatic function, (ix) malabsorption syndromes, (x) malignancy or history of malignancy, (xi) treatment with hypolipidemic, hypotensive, anticoagulant, antiplatelet, or fibrinolytic drugs, (xii) concomitant treatment with drugs that may affect inflammatory processes in the vascular wall (including nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, (xiii) antioxidant therapy, (xiv) ongoing hormonal replacement therapy or oral contraception, (xv) abuse of alcohol, (xvi) smoking cigarettes, and (xvii) poor patient compliance. All subjects expressed informed consent in writing prior to study participation. The study was approved by the Bioethics Commission of the Medical University of Lodz (No 241/06/KB).

Study design

Patients with MS were treated with 5 mg of melatonin 2 hr before bedtime (Melatonina, PF LEKAM Sp. z o.o; Zakroczyn, Polska, Poland) during a 2-month study period. Three control visits were scheduled for subjects: before treatment initiation, and after 1 and 2 months of therapy. During control visits, a clinical examination, measurements of body weight, waist circumference, BP, urine collection, 12-lead electrocardiogram and venous blood sampling for evaluating studied (lipid profile, glucose, CRP, fibrinogen, CAT, GSH-Px, SOD, and TBARS) and safety laboratory parameters (total and differential blood cell count, blood sedimentation rate, alanine and aspartate aminotransferases, electrolytes, bilirubin, creatinine, total proteins) were performed. Blood samples were taken after an overnight fast in a quiet, temperature-controlled room (24–25°C) between 08:00 and 09:00 h (to avoid circadian fluctuations of the parameters studied). The samples were immediately coded so that the person performing the assays was blinded to subject identity and study sequence. Blood pressure was measured between 08:00 and 09:00 h by the same investigator each time, and the mean of the three consecutive measurements was recorded. Compliance was assessed during each visit by tablet counts and was considered satisfactory when the number of tablets taken by a patient ranged from 90% to 100%.

Laboratory tests

The serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, and TG were determined colorimetrically using commercial kits (bio-Mérieux, Marcy l'Etoile, France) 16 hr after the last meal. CRP concentration was measured by latex-particle-enhanced immunoturbidimetric assay. Fibrinogen level was estimated by Clauss method. Glucose level was determined using colorimetric assay. Erythrocytes were specially prepared to assess erythrocyte lipids peroxidation and activity of antioxidative enzymes. Blood collected with anticoagulant (23 mM of citric acid, 45.1 mM of sodium citrate, 45 mM of glucose) was centrifuged at 600 g for 10 min at 4°C to separate plasma and red blood cells. Erythrocytes were washed three times with phosphate-buffered saline. The hematocrit of the final erythrocyte suspensions was made up to about 50%. The peroxidation of erythrocyte lipids was measured by the thiobarbituric acid method by Stocks and Dormandy [17]. Absorbance of color reaction products was measured with a spectrophotometer at 532 nm. Lipid peroxidation was expressed as micromoles of TBARS per gram hemoglobin (Hb). The concentration of Hb was determined using the method by Drabkin [18]; absorbance was measured with a spectrophotometer (540 nm). The activity of CAT [EC 1.11.1.6] in erythrocytes was determined using the Beers et al. method [19], the activity of SOD [EC1.15.1.1] using the Misra et al. method [20], and the activity of GSH-Px [EC 1.11.1.9] using the Rice-Evans method [21].

Data analysis

Results are presented as means ± S.E.M. For normally distributed variables with homogeneity of variance, Student's paired t-test was performed. For those variables that did not meet the homogeneity of variances criterion, a nonparametric Mann–Whitney *U*-test was performed. The multivariate analysis of dependent samples was also used (nonparametric Friedman ANOVA test). All values were tested by Wilcoxon signed ranks test (paired) to find significant differences between the melatonin and control group. Values were considered statistically significant at $P < 0.05$. Statistical analysis was performed using STATISTICA© ver 8.0 PL software (StatSoft Inc., Tulsa, OK, USA).

Results

The study cohort included 30 patients (12 men, 18 women). The control group included 33 healthy volunteers (10 men, 23 women). Patients with MS had significantly higher BMI values and levels of TC, LDL-C, HDL-C, TG, SBP, DBP, glycemia, fibrinogen, and TBARS, and significantly lower activities of SOD, GSH, and CAT compared to healthy

Table 1. Characteristics of patients with metabolic syndrome (MS) compared to values in healthy volunteers

	Controls		Pts with MS at baseline		
	Mean	S.E.M	Mean	S.E.M.	*P* value
N (Male/Female)	33 (10/23)		30 (12/18)		
BMI (kg/m^2)	25.7	2.4	29.0	3.9	<0.01
Total cholesterol (mg/dL)	200.7	30.7	233.4	33.9	<0.001
LDL-C (mg/dL)	120.5	23.4	149.7	26.4	<0.01
HDL-C (mg/dL)	58.6	11.1	48.4	12.6	<0.001
Triglyceride (mg/dL)	99.9	33.4	201.9	106.3	<0.001
SBP (mmHg)	117.2	13.1	132.8	9.8	<0.001
DBP (mmHg)	72.5	6.9	81.7	8.7	<0.01
Glycaemia (mg/dL)	82.7	9.8	91.8	11.8	<0.01
hsCRP (mg/L)	1.8	1.8	3.0	3.5	NS
Fibrinogen (mg/dL)	259.6	89.7	330.3	55.7	<0.001
TBARS (μM/g Hb)	0.3	0.1	0.5	0.2	<0.001
SOD (U/g Hb)	3127.5	616.2	2557.4	620.2	<0.001
GSH-Px (U/g Hb)	19.0	3.0	15.6	2.6	<0.001
CAT (U/g Hb)	370.2	102.5	245.9	46.9	<0.001

controls. CRP levels did not differ significantly between groups (Table 1). One month of melatonin treatment (5 mg/day) resulted in a significant reduction in BMI, SBP, DBP, and TBARS and a significant increase in fibrinogen levels (Table 2). Two months of melatonin treatment (5 mg/day) resulted in a significant reduction in SBP, DBP, LDL, and TBARS levels and a significant increase in CAT activity. A further significant reduction in SBP was observed between values obtained after 1 month versus values obtained after 2 months of melatonin treatment ($P < 0.01$). CRP, fibrinogen, and fasting glycemia concentrations did not change significantly (Table 3).

Discussion

Our study demonstrated that 5 mg/day melatonin administered for 2 months improved SBP, DBP, the serum lipid profile, and antioxidative status. Our studies showed a significant reduction in SBP after 1 month of melatonin treatment, and a further significant decrease was observed after the second month of therapy. A possibility arises that prolonged melatonin administration may have improved outcome. DBP was reduced significantly after 1 month of therapy, while after the second month, significant changes were not observed. After 2 months of melatonin administration, SBP was reduced meanly by a mean of 12.3 mmHg, DBP by 6.5 mmHg.

There is an increasing amount of data on hypotensive effects of melatonin in humans. Interestingly, even low doses of melatonin were proved to lower elevated BP. Administration of melatonin (2.5 mg/day) reduced nocturnal SBP (mean 6 mmHg) and DBP (mean 4 mmHg) in hypertensive subjects [12]. It has been reported that melatonin (10 mg/day) reduced nocturnal BP in subjects with type 1 diabetes mellitus (DM) (SBP – mean 4.4 mmHg, DBP – mean 2.2 mmHg) [22]. It has also been documented that hypertensive patients with nondipping nighttime blood pressure profile have altered melatonin concentrations compared to hypertensive patients with dipping blood pressure profile [23, 24]. Administration of melatonin before sleep time is reported to be effective in lowering BP in the early morning, when the risk of a

Table 2. Effects of 1 month of melatonin administration (5 mg/day) on BMI, serum lipids, BP, glycaemia, inflammatory markers, and oxidative stress parameters

	Patients with metabolic syndrome				
	At baseline		After treatment		
	Mean	S.E.M.	Mean	S.E.M	*P* value
BMI (kg/m^2)	29.0	3.9	28.8	4.0	<0.05
Total cholesterol (mg/dL)	233.4	33.9	220.7	35.4	NS
LDL-C (mg/dL)	149.7	26.4	141.1	33.0	NS
HDL-C (mg/dL)	48.4	12.6	46.5	11.8	NS
Triglyceride (mg/dL)	201.9	106.3	172.8	74.5	NS
SBP (mmHg)	132.8	9.8	126.3	11.5	<0.01
DBP (mmHg)	81.7	8.7	76.9	9.2	<0.05
Glycaemia (mg/dL)	91.8	11.8	92.4	11.4	NS
hsCRP (mg/L)	3.0	3.5	2.8	2.4	NS
Fibrinogen (mg/dL)	330.3	55.7	347.3	65.8	<0.05
TBARS (μM/g Hb)	0.5	0.2	0.4	0.1	<0.05
SOD (U/g Hb)	2557.4	620.2	2774.5	542.0	NS
GSH-Px (U/g Hb)	15.6	2.6	16.7	4.5	NS
CAT (U/g Hb)	245.9	46.9	256.2	46.6	NS

Table 3. Effects of 2 months of melatonin administration (5 mg/day) on BMI, serum lipids, BP, glycaemia, inflammatory markers, and oxidative stress parameters

	Patients with metabolic syndrome				
	At baseline		After treatment		
	Mean	S.E.M.	Mean	M.E.M	*P* value
BMI (kg/m^2)	29.0	3.9	28.8	3.8	NS
Total cholesterol (mg/dL)	233.4	33.9	220.7	31.0	NS
LDL-C (mg/dL)	149.7	26.4	139.9	30.2	<0.05
HDL-C (mg/dL)	48.4	12.6	48.1	11.1	NS
Triglyceride (mg/dL)	201.9	106.3	166.6	53.2	NS
SBP (mmHg)	132.8	9.8	120.5	11.0	<0.001
DBP (mmHg)	81.7	8.7	75.2	7.4	<0.01
Glycaemia (mg/dL)	91.8	11.8	93.3	10.5	NS
hsCRP (mg/L)	3.0	3.5	3.25	3.8	NS
Fibrinogen (mg/dL)	330.3	55.7	316.9	75.3	NS
TBARS (μM/g Hb)	0.5	0.2	0.4	0.1	<0.01
SOD (U/g Hb)	2557.4	620.2	2734.3	379.7	NS
GSH-Px (U/g Hb)	15.6	2.6	17.0	4.6	NS
CAT (U/g Hb)	245.9	46.9	276.8	39.4	<0.01

cardiovascular incident is particularly high [25]. It is postulated that melatonin may reduce BP via a number of mechanisms with the antioxidative effects being of major importance among a number of possibilities [15]. Animal studies suggest that melatonin may exert hypotensive actions via melatonin receptors in the hypothalamus [26], by influencing catecholamine release [27], modulating baroreceptors' response [23], activation of eNOS, and increase in NO synthesis [28]. The rise in eNOS activity may be associated with an increase in calcium concentration in endothelial cells. On the other hand, in vitro studies have shown that melatonin may neutralize NO [29]; however, that effect was observed only for high melatonin concentrations. It is also known that melatonin reacts with peroxynitrite as well as NO [30]. There is clearly a need for further studies to document not only the hypotensive effects of melatonin but also its potential protective effects on the cardiovascular system [31]. Further studies may also bring evidence of possible melatonin interactions with other hypotensive agents. In our studies, we excluded patients treated with antihypertensive drugs. Moreover, it would be interesting to study which patients gain more advantage from melatonin therapy – every patient with arterial hypertension or rather those with nocturnal hypertension.

In our studies, a significant decrease in LDL-C level was observed after 2 months of melatonin treatment. Participants of the study did not respond to a hypolipidemic diet for 3 months before melatonin administration; so, the hypolipidemic effect seems to be because of melatonin treatment. Improvement in lipid profile after melatonin treatment has been observed in previous human studies. Melatonin treatment (1 mg/kg, 30 days) elevated HDL-C levels in peri- and postmenopausal women [32]. The results of animal studies suggest several mechanisms that may be responsible for the hypolipidemic effects of melatonin: decrease in intestinal cholesterol absorption [33], inhibition of cholesterol biosynthesis and LDL-C accumulation [34], interactions with LDL-C receptors [35], or inhibition of fatty acid transport via metabotropic receptors [36]. Animal studies proved that melatonin administered to rats fed with a high-cholesterol diet reduced TC and LDL-C and prevented a decrease in HDL-C [37]. Melatonin ameliorated nonalcoholic fatty liver induced by a high-fat diet in rats that also may affect serum lipids [38]. In ovariectomized rats, melatonin administration prevented an increase in body mass and cholesterol concentration [39]. In our studies, we did not observed changes in body mass after 2 months of treatment, but it is possible that prolonged melatonin supplementation may affect body mass in humans and via this way influence on lipid profile. Some authors claim that the level of melatonin per se is not as important as the melatonin/insulin ratio, and this ratio correlates with lipid profile in patients with MS [40]. It was found that the nocturnal melatonin–insulin ratio correlated negatively with LDL-C and TC and positively with HDL-C levels.

The current study demonstrated that melatonin supplementation increased CAT activity and reduced TBARS level. SOD and GSH-Px were unchanged. However, there was a nonsignificant trend that suggests that prolonged melatonin supplementation may result in significant changes in the studied parameters. Melatonin's antioxidative potential has been documented in hundreds of studies. This has stimulated clinicians to examine melatonin's protective actions in humans. Melatonin administered to patients with hypertension (5 mg/day) increased SOD, CAT, and glutathione reductase activities and reduced malonylodialdehyde (MDA) levels [41]. This confirms our results indicating that melatonin administration may be an adjunct to standard therapy of hypertension, which is one of the criteria of MS. A recent study by the same group demonstrated that supplementation of melatonin (5 mg/day) in patients with DM resulted in an increase in SOD activity and reduction in MDA level [11]. Our results did not prove changes in glucose level, but we excluded patients with DM from the current study. There are several possible mechanisms of inhibition of lipid peroxidation processes by melatonin [30]. Melatonin inhibits oxidation reactions catalyzed by metal ions and scavenges reactive oxygen species, thereby reducing lipid peroxidation. Likewise, melatonin metabolites are all potent antioxidants and also inhibit lipid peroxidation [42]. Melatonin also regulates the

activities of antioxidative enzymes [6]. Melatonin has a role in stimulating glutathione synthesis via elevating activity of γ-glutamylcysteine synthetase or glucose-6-phosphate dehydrogenase (G6PD) [43]. It should be noted that antioxidative influences of melatonin may have a beneficial effect on BP and lipid profile.

In our studies, we treated patients with 5 mg of melatonin, that is a small dose in comparison with doses used in animal studies (5–10 mg/kg), but this is the maximal dose that may be administered to patients according to the producer's registration information. It is possible that higher doses of melatonin may result in further improved outcome.

At an early stage of MS treatment, physicians suggest a nonpharmacological approach as lifestyle modification, a low-fat diet, and physical exercise. Patients who do not respond to these changes are treated with drugs (hypotensive, lipid lowering, and antidiabetic drugs) that may have significant side effects. If additional clinical studies prove the beneficial effects of melatonin in MS subjects, physicians will use this molecule as an adjunct to standard therapy. Melatonin has a high safety profile and it reduces the toxicity of many pharmaceutical agents [44]. Further studies may establish the results of higher doses of melatonin and effects of prolonged melatonin supplementation.

Limitations of the study

The current study has some limitations. The small number of subjects is a limiting factor; so, it may be treated as a pilot study. Another limitation was the research design – a placebo-treated group was not included. We did not perform 24-hr blood pressure monitoring, which may be useful to assess melatonin's effect on BP profile in 'nondippers'. The study was also conducted at a single university hospital; future studies are needed in a representative community-based sample.

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The authors do not have a financial relationship with any commercial entity that has an interest in the subject of this manuscript.

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