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RESEARCH ARTICLE

Antioxidant effect of immediate- versus sustained-release melatonin in type 2 diabetes mellitus and healthy controls

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Abstract

Oxidative damage has been suggested as the primary cause of aging and age-associated diseases including type 2-dependent diabetes mellitus (T2DM) and therefore there is a growing interest in exploring therapeutic potential of antioxidant agents including melatonin. In the present study, we analyzed red blood cell antioxidants and lipid peroxidation after 5 mg/daily immediate-release melatonin treatment of elderly T2DM patients and healthy elderly subjects in comparison with 2 mg/daily sustained-release melatonin treatment of elderly T2DM patients and healthy elderly subjects, to determine the antioxidant effect of different doses and formulations of melatonin in these groups. Our study revealed that there was no significant difference in antioxidant status of red blood cells measured by glutathione concentration and activities of GPx-1, CAT, GR, SOD-1 and MDA levels, after supplementation with 2 mg-sustained release melatonin or with 5 mg-immediate release melatonin, either in T2DM or in healthy elderly subjects. These results suggest that both preparations may exert similar therapeutic effect related to melatonin's action on antioxidant defense system.

Keywords

Aging, antioxidant enzymes, antioxidant supplementation, diabetes mellitus, lipid peroxidation, melatonin, superoxide dismutase

History

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Introduction

Oxidative damage has been suggested as the primary cause of aging and age-associated diseases including type 2-dependent diabetes mellitus (T2DM). Furthermore causative role of oxidative stress in the complications of T2DM has been already recognized and effects of different antioxidants have been tested in these patients (Colak et al. 2005; Fardoun, 2007); however, the rationale for antioxidant supplementation in people suffering from diabetes has also been recently questioned (Loh et al., 2009). There are specific enzymes and low molecular weight substances which eliminate reactive oxygen species and contribute to the redox balance in the cell, however this innate protection decreases with aging and pathologies. Pineal gland product, melatonin, is not only a potent antioxidant agent, but also plays important role for glucose homeostasis and regulation Furthermore, diabetes induces changes in melatonin synthesis in pineal glands and concentrations in peripheral tissues (Stebelová et al., 2007; Peschke et al., 2008).

Recent findings document melatonin's ability to provide cardioprotection at low pharmacological doses (Mukherjee et al., 2010). Melatonin was found to improve cardiovascular function and to exert hypotensive effect due to both its direct antioxidant and receptor-dependent actions, suggesting that the agent may be of therapeutic use, and melatonin treatment may have some beneficial effects in controlling diabetic vascular complications (Simko & Paulis 2007; Kedziora-Kornatowska et al., 2008; Erşahin et al., 2009). Melatonin reduces oxidative stress by acting as a direct free radical scavenger and as an indirect antioxidant when stimulating antioxidant enzymes (Reiter et al., 2007).

Although melatonin is not toxic (Vijayalaxmi & Thomas, 2002), the minimum effective dose approach should also be applied to avoid overtreatment. The doses used for clinical studies on melatonin efficacy vary from 1 mg to 10 daily (Cardinali et al., 2010); however, for diabetes most of the studies were done in animal model. Our group had already reported that 5 mg melatonin daily improved oxidative stress parameters measured in the blood of elderly type 2 diabetic patients (Kedziora-Kornatowska & Szewczyk-Golec, 2009). There is the main concern for fast clearance of the hormone when administrated as immediate-release formulation, which could be circumvented with sustained-release formulation, which provides a melatonin profile in the blood more closely matched to normal physiological release (Wade & Downie, 2008). Melatonin in sustained formulation has been approved as monotherapy for the short-term treatment of primary insomnia characterized by poor Quality of Sleep in patients who are aged 55 or over. Extended release should not affect antioxidant properties of melatonin, however there the effect of sustained release melatonin on antioxidant defense system

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yet needs to be confirmed. Therefore, to overpass this lack of sufficient scientific evidence, the aim of this study was to compare the antioxidant effect of supplementation with low-dose, immediate- and sustained-release melatonin in elderly T2DM patients and healthy elderly subjects. In the present study, we analyzed red blood cell antioxidants and lipid peroxidation after 5 mg/daily immediate-release melatonin treatment of elderly T2DM patients and healthy elderly subjects in comparison with 2 mg/daily sustained-release melatonin treatment of elderly T2DM patients and healthy elderly subjects.

Material and methods

Fifty-two elderly T2DM patients and 43 healthy age and sex-matched controls were recruited to the Department of Gerontology and Clinic of Geriatrics, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz. Patients and controls were blindly assigned to self-administer either 5 mg immediate-release melatonin daily for 30 d ($N = 25$ and $N = 23$, respectively) or 2-mg sustained-release melatonin daily for 30 d ($N = 27$ and $N = 20$, respectively). The sustained-release melatonin preparation is only sold in tablets of 2 mg, which is a daily recommended dose according to marketing authorization holder (Circadin, 2014). Two mg sustained-release melatonin is used to improve sleep quality in patients who are over 55 with primary insomnia. For the same indication, 5 mg/daily immediate-release melatonin is recommended (www.drugs.com) and has been most readily used in previous studies (Ferracioli-Oda et al., 2013). Therefore in our study, we also used 2 mg and 5 mg dose of sustained- and immediate-release melatonin, respectively.

Blood samples were collected twice (a day before melatonin supplementation and after 30 d of the treatment) always in the morning (08:00 h) after overnight fasting into heparinized tubes (6 ml). Reduced glutathione (GSH) concentration was determined spectrophotometrically in the whole blood, according to the method described by Beutler (Beutler, 1971). CuZn superoxide dismutase (CuZn SOD, SOD-1), catalase (CAT), glutathione peroxidase (GPx-1) and glutathione reductase (GR) activities were determined spectrophotometrically in hemolyzed blood samples according to Misra & Fridovich (1972); Beers & Sizer (1952); Paglia & Valentine (1967); Flohé & Günzler (1984) methods, respectively. The malondialdehyde (MDA) concentration was determined using the 2-thiobarbituric acid method, read at 532 nm (Flohé & Günzler, 1984). The concentration of melatonin in serum was determined using the competitive enzyme immunoassay (Melatonin ELISA; IBL, Hamburg, Germany).

The study was approved by the Nicolaus Copernicus University in Toruń Human Ethics Committee and it was conducted under the tenets of the Declaration of Helsinki.

Results

Table 1 shows concentrations of GSH, activities of GPx-1, CAT, GR and SOD-1 and MDA levels in T2DM patients and controls before and after the treatments. The data are presented as mean ± SD. The effect of the treatment was

Table 1. Concentrations of GSH, activities of GPx-1, CAT, GR and SOD-1 and MDA levels in T2DM patients and controls before and after the treatments.

	Melatonin 2 mg – prolonged-release formulation				Melatonin 5 mg – immediate-release formulation			
	DM before $N = 27$	DM after $N = 27$	C before $N = 20$	C after $N = 20$	DM before $N = 25$	DM after $N = 25$	C before $N = 23$	C after $N = 23$
GSH [mmol/l]	2.818 ± 0.4424	2.804 ± 0.4407	3.104 ± 0.2441	3.293 ± 0.6202	2.348 ± 0.3106	2.445 ± 0.4126	2.631 ± 0.2302	2.599 ± 0.2100
% change	−0.485		6.075		4.131		−1.223	
p value	0.9077		0.2125		0.3524		0.6248	
GPx-1 [U/g Hb]	11.76 ± 2.3053	12.88 ± 2.6069	14.30 ± 3.1654	15.14 ± 3.7414	13.60 ± 2.3300	14.76 ± 1.5496	14.30 ± 3.3356	15.28 ± 2.6991
% change	9.534		8.529		5.874		6.826	
p value	0.1005		0.4481		0.0436*		0.2793	
CAT [BU/gHb]	21.46 ± 2.0854	23.02 ± 1.9683	23.60 ± 2.7199	25.51 ± 2.8470	21.79 ± 3.1278	23.29 ± 2.4379	23.29 ± 3.0849	24.21 ± 2.9083
% change	7.240		8.093		6.884		3.945	
p value	0.0001*		0.0364*		0.0646		0.3037	
GR [U/gHb]	56.65 ± 12.940	63.79 ± 15.771	66.98 ± 8.503	73.40 ± 10.196	60.37 ± 7.412	68.66 ± 8.333	64.12 ± 7.322	74.01 ± 6.406
% change	12.609		9.589		13.724		15.424	
p value	0.0747		0.0369*		0.0005*		<0.0001*	
SOD-1[U/gHb]	2130.0 ± 180.04	2384.2 ± 110.49	2226.0 ± 248.29	2395.6 ± 233.24	2740.0 ± 497.97	3191.5 ± 440.58	2568.7 ± 269.01	2846.8 ± 425.51
% change	11.934		16.478		7.617		10.829	
p value	0.0002*		0.0321*		0.0014*		0.0112*	
MDA [μmol/g Hb]	0.228 ± 0.0361	0.199 ± 0.0228	0.228 ± 0.0264	0.207 ± 0.0204	0.268 ± 0.0383	0.209 ± 0.0307	0.283 ± 0.0398	0.242 ± 0.0421
% change	−12.352		−9.011		−21.981		−14.701	
p value	0.0009*		0.0077		<0.0001		0.0015	
Melatonin [pg/ML]	16.51 ± 11.59	28.81 ± 20.19	35.96 ± 16.121	50.65 ± 31.317	19.29 ± 17.152	35.31 ± 26.303	32.60 ± 11.504	46.03 ± 19.018
% change	74.500		40.851		83.048		41.196	
p value	0.0083		0.0699		0.0140		0.0058	

The effect of the treatment was assessed with *t*-test for dependent samples and the Wilcoxon signed-rank test for normally and non-normally distributes variables, respectively. *p* value <0.05 of the effect of the treatment was considered significant, significant values marked with asterisk; % change – percent change in parameter absolute value after the treatment; GSH, reduced glutathione; GPx-1, glutathione peroxidase; CAT, catalase; GR, glutathione reductase; SOD-1, copper zinc superoxide dismutase; MDA, malondialdehyde.

assessed with *t*-test for dependent samples and the Wilcoxon signed-rank test for normally and non-normally distributes variables, respectively. *p* Values of the effect of the treatment and % change in parameter values after the treatment are also given in Table 1.

In this study, we found that melatonin either in 5 mg immediate-release dose or 2 mg sustained-release dose significantly increased SOD-1 activity, both in T2DM patients and healthy controls. Alike, GR activity remained upregulated after both treatment regimens. The enzymes that appeared to be distinctively regulated when compared to two different treatments depends on group, that was CAT. Namely, activity of the enzyme always increased in T2DM patients, however in healthy subjects this effect was observed only after the treatment with 2 mg prolonged-release melatonin.

Based on these preliminary results, we conclude that supplementation with 2 mg-sustained release melatonin and with 5 mg-immediate release melatonin has similar effect on antioxidant status of red blood cells measured by GSH concentration and activities of GPx-1, CAT, GR, SOD-1 and MDA levels, either in T2DM or in healthy elderly subjects. These results suggest that both preparations may exert similar therapeutic effect related to antioxidant action of melatonin.

Discussion

In this study we confirmed modulatory effect of melatonin on antioxidant defense system both in healthy people and T2DM patients. Melatonin is an antioxidant that directly reduces reactive oxygen species such as hydrogen peroxide and peroxynitrite anion, but it also activates and regulates antioxidant enzyme gene expression (Mayo et al., 2002; Kupczyk et al., 2010).

Melatonin regulation of antioxidant enzyme was studied by Mayo (Mayo et al., 2002), who reported that melatonin at physiological serum concentrations was able to increase the mRNA of superoxide dismutase and glutathione peroxidase, and this effect was mediated by a *de novo* synthesized protein. The same study also has suggested that the regulation of antioxidant gene expression is likely to be receptor mediated.

Given that melatonin could be a very powerful antioxidant molecule, that the production of melatonin decreases with age, that the risk for T2DM increase with age and that the free radical effects are involved in the processes of aging and T2DM, it has been suggested that maintaining melatonin at a high level could slow age- and T2DM-related alterations (Mayo et al., 2002; Kupczyk et al., 2010). The study from McMullan et al. (Kupczyk et al., 2010) is considered to be latest in a series of research breakthroughs that suggest melatonin plays an important role in metabolic functions and risk of diabetes. The authors observed that, lower melatonin levels were associated with higher risk of diabetes in women cohort from Nurses' Health Study. Furthermore, those with the very lowest levels of melatonin secretion had 2.17 times the risk for diabetes than those with the highest levels of melatonin, which was 4.27 cases per 1000 and 9.27 cases per 1000, respectively.

A recent study by Kędziora-Kornatowska found that 5 mg of melatonin taken for 30 d before sleep, increased plasma levels of melatonin and the antioxidant enzyme superoxide dismutase, while decreasing lipid peroxidation as measured by MDA concentration in T2DM patients (Kedziora-Kornatowska et al., 2009). The study by Kędziora-Kornatowska also suggests that even low-dose melatonin supplementation exerts antioxidant effects and modulate antioxidant enzyme activities. In our study, we confirm that there was no difference in effects of 2-mg and 5-mg daily melatonin supplementation on redox profile as measured by the concentration of reduced glutathione, activities of antioxidant enzymes (GPx-1, SOD-1, CAT, GR) and concentration of lipid peroxidation product MDA. It is in line with previous research, which has already confirmed that melatonin displays antioxidant properties even at physiological levels (Benot et al., 1999). The clear association between oxidative stress and aging (Rybka et al., 2011) and age-related diseases such as T2DM supports the rationale for implementation of new strategies with classic as well as new antioxidants to treat these conditions and comorbid complications (Johansen et al., 2005). If these antioxidant effects of low-dose melatonin translate into therapeutic outcomes, yet needs to be elucidated, which opens frontiers for further research. The prospective research should look at the effects of low-dose melatonin on insulin sensitivity and glucose metabolism to help identify the mechanism by which melatonin influences the development of diabetes.

Declaration of interest

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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