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Effects of melatonin and zinc on oxidative stress in poorly controlled type 2 diabetic patients treated with metformin

***Ahmed Rahma Abu-Raghif , ** Haitham M. Kadhim, ***Abdul-kareem H. Abd , ****Saad Abdul-Rehman Hussain**

,**, Department of Pharmacology, College of Medicine, University of Al Nahrain, Baghdad, Iraq**
****** Department of Pharmacology, College of Pharmacy, University of Baghdad, Baghdad, Iraq**

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Abstract

Background: Glycemic control and prevention of secondary complications are the most important goals of using pharmacologic treatment of diabetes mellitus (DM).The administration of antioxidants such as melatonin and zinc may improve tissue responses to insulin and increase the efficacy of drugs, e.g. metformin, which act through this pathway. This project was designed to evaluate the effects of melatonin and zinc on the oxidative stress status in type 2 DM patients poorly controlled with metformin. Patients and methods: A placebo-controlled, double-blind clinical trial was performed in which 46 type 2 diabetic patients were selected and allocated into three groups. These groups were treated with single daily oral doses of both 10 mg of melatonin and 50 mg of zinc acetate alone: in addition to the regularly used metformin or placebo, given at bedtime for 90 days. Plasma Malondialdehyde (MDA); plasma glutathione (GSH); and plasma zinc and copper levels were measured before initiating the treatments (zero time) and after 30 and 90 days of treatment. Results: Daily administration of melatonin and zinc effectively normalizes the impaired antioxidant status, and significantly elevates serum zinc levels after 30 and 90 days compared to placebo treated group; the addition of this treatment regimen in combination with metformin improved the tissue responses to this oral hypoglycemic agent. Conclusion: the combination of melatonin and zinc acetate, when used alone or in combination with metformin, has a significant decrease in lipid peroxidation and improvement in antioxidant status , and this effect can be related to several mechanisms in type 2 DM patients.

تأثيرات مادتي الميلاتونين و الخارصين على فرط الإجهاد ألتأكسدي في المرضى المصابين بداء السكري النوع الثاني من ضعيفي الاستجابة لعقار المتفورمين

احمد رحمة ابو رغيف هيثم محمود كاظم عبدالكريم حميد عبد سعد عبدالرحمن حسين

الملخص

خلفية الدراسة: يعتبر تنظيم مستوى الكلوكوز في الدم ومنع التعقيدات الثانوية من أهم الأهداف التي نتوخاها من استخدام العقاقير لعلاج داء السكري . إن استخدام مضادات أكسد ة فعالة مثل الميلاتونين و الخارصين قد يؤدي إلى تحسين استجابة الأنسجة للأنسولين والى زيادة في فعالية العقاقير المستخدمة لهذا الغرض مثال ذلك عقار المتفورمين ، والتي تعمل من خلال هذا الاتجاه . تم تصميم هذه الدراسة لتقييم تأثير الميلاتونين و الخارصين في السيطرة على حالة فرط الإجهاد التأكسدية المصاحبة لداء السكري لدى مرضى النوع الثاني من داء السكري ضعيفي الاستجابة للعلاج بمادة المتفورمين.

المرضى والطرق : أجريت الدراسة بالطريقة العمياء المزدوجة التي تعتمد على المقارنة مع تأثير مستحضر تهدئة (placebo) على 46 مريضاً بالنوع الثاني من داء السكري والذين تم اختيارهم وتوزيعهم على ثلاثة مجاميع تم علاجهم بإحدى الطرق التالية:

- مجموعة تم علاجها بجرعة يومية عن طريق الفم تحتوي 10 ملغم ميلاتونين مع 50 ملغم خلات الخارصين فقط ولمدة 90 يوماً.
- مجموعة تم علاجها بجرعة يومية عن طريق الفم تحتوي على 10 ملغم ميلاتونين مع 50 ملغم خلات الخارصين بالإضافة إلى الجرعة المعتادة من مادة المتفورمين ولمدة 90 يوماً.
- مجموعة ثالثة تم علاجها بجرعة يومية عن طريق الفم من مستحضر تهدئة (placebo) بالإضافة إلى جرعة المتفورمين المعتادة ولمدة 90 يوماً.

تم قياس المعايير التالية : مستوى مالون دايالديهايد (MDA) في الدم و مستوى الغلوتاثايون (GSH) و مستوى الخارصين والنحاس في الدم لدى جميع المشتركين في الدراسة قبل البدء بالعلاج وبعد 30 و 90 يوماً أثناء العلاج.

النتائج: أظهرت النتائج بان العلاج اليومي بمادة المي لاتونين و الخارصين لوحدهما ، بالإضافة إلى إن إضافة هذا النظام العلاجي إلى الطريقة المتبعة باستخدام الميلاتونين أدى إلى تحسن في استجابة الأنسجة للعلاج (المتفورمين) بهذا الخصوص مقاربة باستخدام المتفورمين لوحده أو باستخدام جرعة إل placebo.

الاستنتاج: من خلال ما تم عرضه من نتائج يمكن الاستنتاج بأن استخدام الميلاتونين و الخارصين لوحدهما أو مع المتفورمين يؤدي إلى تقليل أكسدة الدهون المصاحبة لداء السكري لدى مرضى النوع الثاني من هذا الداء بشكل معتد به وكذلك تحسن الحالة المضادة للأكسدة وهذا التأثير قد يعزى إلى عدة ميكانيكيات في المرضى المصابين بداء السكري النوع الثاني.

Introduction

The importance of a process such as oxidative stress (OS) in pathology is dependent on both its sources and its targets. From that point of view, diabetes is an exquisite candidate to study the biological impact of OS and the potential efficacy of its correction [(1)]. Non-enzymatic, free radical–mediated oxidation of biological molecules, membranes and tissue is associated with a variety of pathological events such as cancer, ageing and diabetes mellitus [(2)]. In the latter, OS seems primarily due to both an increased plasma free radical concentration and a sharp reduction in antioxidant defenses [(3)].Among the causes of enhanced free radical production; hyperglycemia and hyperinsulinemia seem to play a major role [(4)]. Evidence that OS is present in diabetes originates from the frequent observation that both reactive oxygen species (ROS) and antioxidants are increased. The later is logically rather seen in early stage of diabetes and should be interpreted as a tentative compensation of cells against increasing OS [(5)]. Oral intake of large amounts of glucose in animals increases thiobarbituric reactive substances (TBARS) and reduces the activity of hepatic enzymes susceptible to thiol group oxidation [(6)]. In humans, OS is also seen in post-prandial periods in normal individuals, but diabetic patients are unable to compensate for the increased ROS [(7)]. This increase may be attributed to acute effects of high glucose and/or lipids. Type 2 diabetics exhibit increases in TBARS and reduction in catalase activity, but surprisingly, correlation was found between TBARS and level or duration of hyperglycemia [(5)]. Plasma glutathione (GSH) levels are decreased and oxidized purines increase; illustrating DNA damage [(7)]. Interestingly, even in a healthy population, variations in insulin sensitivity are related to lipid hydroperoxides levels and reduced catalase and vitamin E levels [(8)]. Again in the general population, various markers of glucose metabolism and of insulin resistance were associated with OS [(9)]. Melatonin, chemically known as N-acetyl-5-methoxy tryptamine, is a simple methylated and N-acetylated product of serotonin, produced by the pineal gland and some other tissues [(10)]. Its production in mammalian species,

including humans, is regulated by the photoperiodic environment with the daily period of light being associated with low melatonin production, whereas during darkness its synthesis and secretion increase markedly, so it is produced and released in circadian rhythm in all vertebrates studied [(11)]. Both *in vitro* and *in vivo* studies [(12)], melatonin has shown to be a potent scavenger of the highly toxic hydroxyl radical and other oxygen centered radicals, suggesting that it has action not mediated by receptors[(13)], and it seems to be more effective than other known antioxidants (glutathione and vitamin E) in protecting against oxidative damage. Therefore, melatonin may provide protection against diseases that cause degenerative or proliferative changes by shielding macromolecules, particularly DNA, from such injuries. However, this antioxidant activity requires concentrations of melatonin to be much higher than night peak concentrations [(14)]. Thus, the antioxidant effects of melatonin in humans probably occur only at pharmacological concentrations. It has been demonstrated that melatonin show a marked protective effect against oxidative stress and severity of diabetes induced by streptozocin in rats, this confirm the powerful antioxidant action of this pineal indole, and the importance of the severity of oxidative stress to maintain hyperglycemia and protein glycosylation, two pathogenetic cornerstones indicative of diabetic complications [(15)]. Accordingly, melatonin, when used in high pharmacological doses alone or with other dietary supplements like zinc, may have a powerful improving role in the control of DM and its related complications; during treatment with oral hypoglycemic agents like biguanides. Zinc stimulates glucose transport through a post-insulin receptor mechanism. On the other hand, zinc may be involved in the pathway of insulin signaling through the inhibition of certain membrane-associated tyrosine phosphatase activity, which is known to antagonize insulin effect at receptor level [(16).] Zinc also stimulates both, membrane localization and activity of certain isoform of protein kinase-C appeared to be important in insulin signaling [(17)] . This study was designed to evaluate the possible role of using melatonin in regular daily pharmacological doses (10 mg/day) in association with zinc acetate in recommended daily allowance (RDA) levels, in the improvement of oxidative stress status, and plasma zinc level of type 2 DM in patients treated with biguanides, but with poor control.

Patients and methods

This study was performed on 46 patients with type 2 DM at the Specialized Center for Endocrinology and Diabetes, Al-Rusafa Directorate of Health, Baghdad, and the study protocol was approved by its scientific committee. Twenty five males and 21 females with an age range of 40–64 yr (49.1 ± 6.0) and disease duration of 4.2 ±3.1 yr were included. All the selected patients had no other marked pathologic disorders such as hypertension and ischemic heart diseases as revealed by the clinical investigation. Thirty three patients were treated previously with maximal dose of metformin (Menarini International, Frienze, Italy) (2550 mg/day, i.e. 850mg t.i.d.) and kept on dietary control, but with poor glycemic control as evidenced by abnormal values of fasting plasma glucose and glycated hemoglobin. These patients were carefully evaluated while they were on their already established treatment program for DM control for 2 wk before being included in three groups:

1-Group (A) included 15 patients (eight men and seven women) treated with placebo in capsule form in addition to the oral hypoglycemic

agent (metformin 2550 mg/day) and under dietary control for 3 months.

2-Group (B) included 18 patients (11 men and seven women) treated with a combination of 10 mg of melatonin (Rupal Chemicals Ltd, Tarapur, India) and 50 mg of zinc acetate (Fluka-Garantie, Buchs, Switzerland), given as single daily doses in a capsule form, in addition to the oral hypoglycemic agent (metformin 2550 mg/day) and under dietary control for 3 months.

3-Group (C) included the remaining 13 DM patients (seven men and six women) who were newly diagnosed and maintained for 2 wk on dietary control program only. They were then treated with 10 mg of melatonin and 50 mg of zinc acetate as single daily doses as capsule form in addition to a dietary control program for 3 months.

4-Seventeen healthy subjects (nine men and eight women) in the same age range as that of patients were selected and served as **controls**.

All patients were selected according to the following criteria: they did not have other associated chronic diseases such as liver or kidney disorders and no cardiovascular complications. Diabetic women who were pregnant or breast-feeding were excluded. They were not on insulin therapy or on antioxidant drugs including aspirin, or on any associated drugs. After 12 hr of fasting, blood samples (10 ml) were collected from all subjects via venous puncture before starting drug treatment (zero time sample) and then after 30 and 90 days of treatment to follow the changes in the studied parameters. Blood samples were collected in citrate-containing tube and centrifuged at 10 g for 10 min at 4_C; after centrifugation and isolation of cellular fraction, the plasma fraction was stored frozen until analysis performed. Plasma Malondialdehyde (MDA) was measured according to the standard method of stocks and Dormandy (1971) [(18)], which is modified by Gilbert *et al* (1984) [(19)]. Plasma Glutathione contents (measured as total sulfhydryl groups) were measured according to the method of Godin *et al* (1988) [(20)]. Plasma zinc level sample was diluted 5 folds with deionized water and was aspirated into the atomic absorption spectrophotometer[(21)]. In case of copper determination, the plasma sample was diluted with an equal volume of deionized water and directly aspirated into the atomic absorption instrument [(22)]. Paired t-test and ANOVA were used to examine the degree of significance, and a value of $P < 0.05$ was considered significant.

Results

The data presented in table 1 clearly showed that all selected DM patients demonstrate significantly higher levels of MDA production (marker of lipid peroxidation) compared to controls. Treatment with melatonin and zinc resulted in significant decrease in serum MDA levels after 30 days (17% and 20% respectively) and after 90 days (group B and C), compared to baseline values. No effects were observed for placebo formula in this respect (group A), and the effects of the melatonin and zinc formula were found to be comparable in their effects and significantly different compared to placebo formula. Table 1 indicated also severe depletion of glutathione in the serum of all selected diabetic patients, an event that can not be corrected by treatment with placebo formula (group A). However, treatment with melatonin and zinc resulted in significant elevation in serum GSH levels in group B and C DM patients after 30 days (14% and 28% respectively) and after 90 days (54% and 81% respectively) compared to the pre-treatment values. Treatment with the tested formula alone seems to produce significantly higher levels of GSH elevation in group (C) patients compared to metformin and the tested formula (group B), and both types of treatment were found significantly more effective compared to placebo formula

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Table (1): Effects of daily treatment with 10 mg melatonin and 50 mg zinc acetate on serum levels of malondialdehyde and glutathione in type 2 diabetic patients.

Group	Duration	MDA µmol/L	GSH µmol/L
Control N = 17	--------	0.913 ± 0.038	0.37462 ± 0.005
Group A N = 15	base line	2.09 ± 0.092 [a *]	0.196 ± 0.010 [a *]
	30 days	1.85 ± 0.136 [b]	0.200 ± 0.013 [a]
	90 days	2.05 ± 0.100 [a *]	0.1935 ± 0.015 [a]
Group B N = 18	base line	2.06 ± 0.085 [a *]	0.195 ± 0.013 [a *]
	30 days	1.71 ± 0.084 [b]	0.223 ± 0.020 [b *]
	90 days	1.24 ± 0.048 [c]	0.300 ± 0.026 [c]
Group C N = 13	base line	2.14 ± 0.08 [a *]	0.180 ± 0.011 [a *]
	30 days	1.72 ± 0.076 [b]	0.230 ± 0.013 [b]
	90 days	1.43 ± 0.06 [c]	0.326 ± 0.016 [c]

-Group(A): patients treated with placebo + metformin (850mg tab. t.i.d).
-Group(B): patients treated with the formula 10 mg melatonin and 50 mg zinc acetate per day in capsules + metformin (850mg tab. t.i.d).
-Group(C):patients treated with 10mg melatonin and 50 mg zinc acetate per day in capsules without metformin.
-Control: healthy subjects without any medication.
-Results were represented as mean ± standard error.
-N = number of subjects.
-Results with non-identical superscripts (a, b, c) among the same group were considered significantly different (P<0.01).
- * = Significant difference from control, P<0.05.

Table (2):- showed that serum copper levels in all selected diabetic patients were significantly higher than those observed in control (P<0.01) and treatment with placebo did not produce any significant changes after 90 days of treatment. However, treatment with the tested formula in both groups (A and B) resulted in significant reduction in serum copper after 30 days (11% and 15% respectively) and after 90 days (24% and 25% respectively). Table (2) also indicated that all selected diabetic patients are presented with lower serum zinc levels compared to controls (P<0.01), and placebo treatment did not produce significant changes in zinc levels during 90 days treatment. Table 2 also indicated that, treatment with melatonin and zinc produced significant increase in serum zinc levels in DM patients after 30 days (4% and 10% respectively) and after 90 days (21% and 32% respectively) compared to pre-treatment levels. In regarding to the serum level of copper and zinc, treatment

with melatonin and zinc significantly improves the Cu/Zn ratio, which seems to be severely impaired in DM patients (Table 2).

Table (2): Effects of daily treatment with 10 mg melatonin and 50 mg zinc acetate on serum levels of zinc and copper in type 2 diabetic patients.

Group	Duration	Serum Cu µg/dl	Serum Zn µg/dl	Cu/Zn ratio
Control N = 17	-------	106.81 ± 1.65	99.77 ± 1.32 [a]	1.07
Group A N = 15	base line	157.00 ± 5.94 [a *]	65.25 ± 2.66 [a *]	2.40
	30 days	158.75 ± 4.716 [a]	64.00 ±1.75 [a]	2.48
	90 days	162.62 ± 2.463 [a]	62.43 ± 2.38 [a]	2.60
Group B N = 18	base line	169.88 ± 4.322 [a*]	73.60 ± 1.87 [a *]	2.30
	30 days	152.0 ± 5.00 [b]	76.45 ± 2.22 [b]	1.98
	90 days	129.77 ± 2.58 [c]	89.18 ± 2.58 [c]	1.45
Group C N = 13	base line	166.285 ± 6.9 [a *]	77.63 ± 2.33 [a *]	2.10
	30 days	150.85 ± 7.4 [b]	85.07 ± 1.88 [b]	1.70
	90 days	124.28 ± 7.95 [c]	102.81 ± 3.45 [c]	1.20

-Group(A): patients treated with placebo + metformin (850mg tab. t.i.d).
-Group(B): patients treated with the formula 10 mg melatonin and 50 mg zinc acetate per day in capsules + metformin (850mg tab. t.i.d).
-Group(C):patients treated with 10mg melatonin and 50 mg zinc acetate per day in capsules without metformin.
-Control: healthy subjects without any medication.
-Results were represented as mean ± standard error.
-N = number of subjects.
-Results with non-identical superscripts (a, b, c) among the same group were considered significantly different ($P<0.01$).
- * = Significant difference from control, $P<0.05$.

Discussion

Diabetes increases oxidative stress in many organs such as liver, kidney and heart [(3)]. Melatonin is known to be an endogenous free radicals scavenger and an efficient antioxidant; it detoxifies a variety of free radicals and reactive oxygen intermediates including hydroxyl radicals, singlet oxygen, superoxide, nitric oxide [(23)], and peroxynitrite anion [(24)]. In the present study, diabetic patients showed an increase in the serum MDA levels associated with significant depletion of serum GSH levels (Table 1). Experimental study in STZ-induced diabetes reveals an increase in lipid peroxidation, which is one of the deleterious effects of oxidative stress [(25)]. Meanwhile, treatment with melatonin may effectively normalizes the impaired antioxidant status, an effect which may

be useful in delaying the emergence of complications like retinopathy, nephropathy and neuropathy, as a result of imbalance between free radicals production and efficiency of the antioxidant systems [26] It has been shown that melatonin increases antioxidant enzyme activity by inducing their gene expression [27]. The increase in GSH levels induced by melatonin may function to reduce oxidative stress and to regulate cellular growth [23]. Fauer and coworkers in 1995 demonstrated that, in people with type 1 DM receiving 30 mg zinc as zinc gluconate for three months, there is a significant decrease in lipid peroxidation and improvement in antioxidant status [28], and this effect can be related to several mechanisms, among them the involvement of zinc-metallothionein complexes in the protection of β-cells against immune-related free radicals attack [29]; and zinc can also act to protect sulfhydryl groups against oxidation in association with inhibition of free radical production through Haber Weiss cycle by competing with copper and iron [30]. It is clearly showed that patients with type 2 DM showed significant zinc deficiency [31]. The results in(Table 2), clearly showed this picture which may be due to abnormal excretion of high urinary zinc and/or malabsorption, also (Table 2) showed that patients with type 2 DM have significantly higher levels of serum copper, compared with normal controls a result which is compatible with that obtained by Zargar and coworkers [32]. Treatment with melatonin and zinc acetate significantly elevates serum zinc levels after 30 and 90 days compared to placebo treated group (Table 2). Many factors are known to influence absorption of zinc, among them dietary factors [33]. High phytate and iron intake have negative effects, while proteins, amino acids and other low molecular weight ions, such as organic acids like citrate, picolinate have a positive effects on zinc absorption, and have been used as salts of zinc in its supplement formulation[34]. On these basis, Ueda and coworkers [35], showed that new Zn (II) complexes with 2-picolinamide and 6-methyl-2 picolinmethyl amide demonstrated high insulinomimetic activity *in vitro* and *in vivo*. Yoshikawa and coworkers [36] showed that Zn (II) complexes of

α-amino acids and their derivatives with Zn (N_2O_2) coordination mode, exhibits excellent blood glucose lowering effect, which in turn improves the diabetic state of the animals. So, improvement of glycemic control may indirectly re-balance the trace metal states through establishment of normal homeostasis. In conclusion according to the results presented in this study one can conclude that melatonin and zinc, when used in combination, could effectively normalizes the impaired antioxidant status, an effect which may be useful in delaying the emergence of complications like retinopathy, nephropathy and neuropathy, as a result of imbalance between free radicals production and efficiency of the antioxidant systems.

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