THE EFFECT OF MELATONIN AGAINST GASTRIC OXIDATIVE STRESS AND DYSLIPIDEMIA IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Objective. The aim of this study was to evaluate the possible protective effects of MT against gastric oxidative stress and dyslipidemia in streptozotocin (STZ) - induced diabetic rats.

Methods. Forty male Wistar rats were randomly divided into five groups: control, diabetic, MT 5 mg/kg+ STZ, MT 10 mg/kg+ STZ and MT 20 mg/kg+ STZ. STZ (60 mg/kg) was intraperitoneally (ip) injected as a single dose for diabetes induction. One week after STZ administration, MT was injected daily as ip for 14 days. The levels of malondialdehyde (MDA), total thiol and glutathione, as well as superoxide dismutase (SOD) and catalase activities were measured in gastric tissue. Serum concentrations of triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) were also determined.

Results. Serum glucose significantly increased in diabetic group compared to control group. STZ induced a significant decrease in gastric tissue levels of total thiol, glutathione, catalase and SOD activities and a significant increase in MDA concentration. In diabetic rats, serum TG, LDL and TC were significantly higher and HDL was significantly lower than in the control group. Treatment of diabetic rats with MT caused a significant increase in gastric total thiol content and glutathione concentration as well as SOD and catalase activities. Gastric MDA concentration and serum LDL, TG and TC were significantly lower in MT-treated groups when compared with diabetic group.

Conclusion. These data suggested that MT has a therapeutic effect on gastric oxidative damage and dyslipidemia induced by diabetes that possibly may be due to its antioxidant effects.

Key words: Melatonin, diabetes mellitus, streptozotocin, oxidative stress, Dyslipidemia.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder with multiple etiologies; it is characterized by chronic hyperglycemia with disturbed metabolism of carbohydrate, protein and fat due to deficiency of insulin secretion, disturbance in its action, or both. Long-standing injury, malfunction and failure of many organs are some of DM complications (1). Many of people from infancy to old ages, with different races and socio-economic ranks may be affected by DM worldwide (2). It has been shown that hyperglycemia, the most common feature in both type 1 and type 2 diabetes, generates the reactive oxygen species (ROS) and changes the redox balance and thus contributes to oxidative stress (3). The most possible cause of redox imbalance is disability of antioxidant defense systems for scavenging of free radicals. During diabetes the main source of free radicals is glucose oxidation, so that enediol form of glucose during a transition-metal dependent reaction is oxidized to an enediol radical anion and finally a lot of reactive free radicals are produced (4). It has also been reported that hyperglycemia, via a superoxide-dependent reaction, increases the LDL lipid peroxidation and generates free radicals (5). The altered antioxidant activity plays an important role in the pathogenesis of diabetes (6). An excess of ROS in mitochondria disturbs antioxidant defenses, causing bioenergetic failure and metabolic complications (7). Melatonin (MT) is a biogenic amine hormone secreted from the pineal gland (7), as well as gastrointestinal tract (GIT); MT receptors are found throughout the GIT in vertebrates (8). The amounts of MT in GIT are higher than those from pineal (9,7). Recent studies have shown that MT demonstrates

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hypoglycemic and hypolipidemic effects, similar to insulin (10). Also, it has been reported that MT, as an amphipathic antioxidant, enters the mitochondria in a dose- and time-dependent manner (11). Therefore, the aim of the present study was to investigate the possible protective effects of MT against oxidative stress and dyslipidemia in STZ-induced diabetic rats.

MATERIALS AND METHODS

Animals and experimental protocol

Forty male Wistar rats (10 weeks old, weighing 250 ± 20 g) were maintained at $22 \pm 2^\circ\text{C}$ and 12 h light/dark cycle. All experiments were performed under the authority of the Mashhad University of Medical Sciences and the norms of international animal ethics were followed. The animals were randomly divided into five groups of 8 in each as follows: group 1: control, group 2: diabetic, group 3: MT 5 mg/kg+ streptozotocin (STZ), group 4: MT 10 mg/kg+ STZ, group 5: MT 20 mg/kg+ STZ. Diabetes was induced in all diabetic groups by a single dose intraperitoneal (ip) injection of STZ (60 mg/kg). To confirm hyperglycemia, 72 hours after STZ injection, blood glucose were measured using a glucometer (Clever check, TD-4230) from tail vein blood sample; glucose levels higher than 300 mg/dL were considered to be diabetic. One week after STZ injection (10th day of study), the animals in groups 3-5 were treated respectively with daily ip administration of 5, 10 and 20 mg/kg of MT for 2 weeks. At the end of the experiment, blood glucose was measured again and serum samples were collected from the orbital sinus and were kept in -20°C for lipid profile measurement. Then, all animals were sacrificed and gastric tissues were quickly removed and conserved at -20°C for oxidative stress assessment.

Malondialdehyde (MDA) measurement

MDA as an index of lipid peroxidation reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance to produce a red colored complex. It has a peak absorbance at 535 nm. 15g TCA, 0.375g TBA and 2mL HCl were mixed and 2 mL of this mixture was added to 1 mL of serum or kidney homogenate and the mixture was heated for 50 min in a boiling water bath. After cooling, the mixture was centrifuged at 1000 rpm for 10 min. The absorbance (A) of colored layer was read at 535 nm. MDA concentration was calculated from the following equation (12).

$$C (M) = A / 1.56 \times 10^5$$

Total thiol content assay

Total -SH groups were measured using DTNB as the reagent. This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm. 1 mL, Tris-EDTA buffer was added to 50 μL of serum or kidney homogenate and absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 μL DTNB reagents (10 mM in methanol) were added to the mixture and after 10 min, the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation (13):

$$\text{Total thiol concentration (mM)} = (A2 - A1 - B) \times 1.07 / 0.05 \times 13.6.$$

Assessment of antioxidant enzymes

Superoxide dismutase (SOD) activity in stomach tissue was determined by the method of Madesh (14). In a colorimetric assay, the SOD activity was measured at 570 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of MTT reduction by 50%. The results were shown as unit per milligram protein. Catalase activity was measured according to the method of Aebi with hydrogen peroxide (30 mM) as the substrate. One unit of catalase activity is determined as the micromoles of the hydrogen peroxide consumed per milligram of protein sample (15).

Reduced Glutathione (GSH) Measurement

GSH concentration was determined via procedure of Ellman (13). Briefly, 0.5 mL of tissue homogenate, 1.5 mL 0.15 M KCl and 3 mL deproteinized solution were blended. Samples were centrifuged at 3000 rpm for 10 minutes then the supernatant was collected and 2mL phosphate solution plus 0.5 mL DTNB was added to the 0.5 mL supernatant; the absorbance was read at 412 nm then compared to glutathione standards (16).

Lipid profile assessment

Serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density protein (LDL) were measured by commercial laboratory kits (Pars Azmoon, Tehran, Iran). Levels are reported in milligrams per deciliter of blood, or mg/dL (17).

Statistical analysis

The data were expressed as mean $\pm$ SEM. One-way ANOVA was run followed by a post hoc Tukey

comparison test; statistical significance was considered as $p < 0.05$.

RESULTS

As shown in Figure 1, the blood glucose level in diabetic group significantly increased when compared with control group ($p < 0.001$). Treatment of diabetic rats with MT at doses of 10 and 20 mg/kg caused a significant decrease in blood glucose in comparison with diabetic rats ($p < 0.001$) (Fig. 1).

Effect of melatonin on gastric oxidative stress biomarkers in diabetic rats

Gastric MDA concentration

As shown in Figure 2, the MDA levels in diabetic group significantly increased compared with control group ($p < 0.001$). However, treatment of

diabetic rats with MT at doses of 10 and 20 mg/kg caused a significant decrease the lipid peroxidation compared to diabetic rats ($p < 0.001$) (Fig. 2).

Gastric total thiol content

In diabetic rats, total thiol concentration was significantly lower than those of control group ($p < 0.001$) (Fig. 3). Administration of MT at doses of 5, 10 and 20 mg/kg significantly increased the gastric total thiols in comparison with diabetic group ($p < 0.05$, $p < 0.05$ and $p < 0.01$ respectively) (Fig. 3).

Gastric GSH levels

GSH levels in diabetic group showed a significant decrease when compared with control animals ($p < 0.001$) (Fig. 4). Two weeks administration of MT at doses of 10 and 20 mg/kg significantly increased the gastric GSH levels when compared with diabetic group ($p < 0.001$) (Fig. 4).

Figure 1. Blood glucose level in all experimental groups. Values are the Mean $\pm$ SEM. The data were analyzed using one-way ANOVA & post hoc Tukey.

*** $p < 0.001$ compared to control group.

+++ $p < 0.001$ compared to diabetic group.

Figure 2. MDA concentration in all experimental groups. Values are the Mean $\pm$ SEM. The data were analyzed using one-way ANOVA & post hoc Tukey.

*** $p < 0.001$ compared to control group.

+++ $p < 0.001$ compared to diabetic group.

Figure 3. Total thiol concentration in all experimental groups. Values are the Mean $\pm$ SEM. The data were analyzed using one-way ANOVA & post hoc Tukey.

*** $p < 0.001$ compared to control group.

+ $p < 0.05$ compared to diabetic group.

++ $p < 0.01$ compared to diabetic group.

Figure 4. Reduced Glutathione (GSH) levels in all experimental groups. Values are the Mean $\pm$ SEM. The data were analyzed using one-way ANOVA & post hoc Tukey.

*** $p < 0.001$ compared to control group.

+++ $p < 0.001$ compared to diabetic group.

Figure 5. Superoxide dismutase (SOD) activity in all experimental groups. Values are the Mean $\pm$ SEM. The data were analyzed using one-way ANOVA & post hoc Tukey.

* $p < 0.05$ compared to control group.

+ $p < 0.05$ compared to diabetic group.

++ $p < 0.01$ compared to diabetic group.

Gastric antioxidant enzymes activities

As shown in Figures 5 & 6, SOD and catalase activities significantly decreased in diabetic group in comparison to control group ($p < 0.05$ and $p < 0.001$ respectively). MT administration to diabetic animals caused a significant increase in SOD activity compared with diabetic rats ($p < 0.05$, $p < 0.01$ and $p < 0.01$ respectively) (Fig. 5). However, treatment of diabetic rats with the highest dose of MT (20 mg/kg) significantly increased the gastric catalase activity when compared with diabetic group ($p < 0.001$) (Fig. 6).

Effect of melatonin on serum lipid profile in diabetic rats

In diabetic group, serum levels of TG, TC and LDL showed a significant increase (all $p < 0.001$), but a significant decrease was seen in serum HDL concentration compared with control group ($p < 0.001$) (table 1). However, in MT- treated rats the amounts of TG, TC and LDL were significantly lower than those of diabetic group (all $p < 0.001$) (Table 1).

DISCUSSION

In the present study, our results showed that MT significantly improved hyperglycemia, gastric

Figure 6. Catalase activity in all experimental groups. Values are the Mean $\pm$ SEM. The data were analyzed using one-way ANOVA & post hoc Tukey.

*** $p < 0.001$ compared to control group.

+++ $p < 0.01$ compared to diabetic group.

oxidative damage and alterations in serum lipid profile in STZ-induced diabetic rats. STZ is a widely used chemical agent for induction of type 1 diabetes because of its ability to destroy the pancreas β cells and thus promotion of insulin deficiency. Our results in agreement with previous studies revealed that serum glucose level significantly increased by STZ administration, confirming diabetes induction. In the current study, diabetes was accompanied by gastric oxidative stress and serum lipid abnormalities. We recently also reported the ameliorating effect of MT on gastric motility and secretion in diabetic rats (18). Diabetes mellitus is a metabolic complication associated with hyperglycemia and several endogenous organs insufficiencies. Gastric tissue is highly affected by free radicals generated during diabetes (19). Several evidences indicate that oxidative stress plays a pivotal role in pathogenesis of diabetes mellitus (20). High level production of free radicals during diabetes and the simultaneous dysfunction of antioxidant defense systems can lead to cellular damage and increased lipid peroxidation (20). Among the antioxidant enzymes, SOD and catalase play a principal role in protecting cells against harmful free radicals, although their activities reduce during diabetes (21). It has been proposed that hyperglycemia via the free radicals generation interferes with antioxidant defense systems (22). Our

Table 1. The effect of melatonin on serum lipid profile of the experimental groups

	Control	Diabetic	MT 5mg/kg+STZ	MT 10mg/kg+STZ	MT 20mg/kg+STZ
TG (mg/dL)	77.16 $\pm$ 1.33	101 $\pm$ 3.26***	73.68 $\pm$ 1.74###	70.15 $\pm$ 1.99###	70.99 $\pm$ 2.00###
TC (mg/dL)	62.77 $\pm$ 1.35	90.12 $\pm$ 2.42***	59.78 $\pm$ 1.45###	58.94 $\pm$ 1.24###	60.46 $\pm$ 1.99###
LDL (mg/dL)	6.54 $\pm$ 1.12	41.64 $\pm$ 2.6***	22.31 $\pm$ 2.39###	20.61 $\pm$ 2.57###	10.7 $\pm$ 1.6###
HDL (mg/dL)	40.8 $\pm$ 1.44	28.22 $\pm$ 1.92***	19.32 $\pm$ 1.61###	24.29 $\pm$ 2.18	31.89 $\pm$ 1.64

MT: melatonin, STZ: streptozotocin, TG: triglycerides, TC: total cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein.

All data were expressed as mean $\pm$ SEM (n=8 in each group). *** $p < 0.001$ compared to control group. ### $p < 0.01$, #### $p < 0.01$ compared to diabetic group.

results are in line with these findings. GSH plays an important role in glucose performance as a stimulant for insulin secretion (23) and the GSH level is relevant to the balance between the oxidation and the reduction cycles of glutathione (24). Therefore, abnormal GSH levels in the diabetic animals of the present study might be attributed to changes in the GSH metabolism in this cycle following increased blood glucose levels (25). In our study, MT significantly decreased serum glucose level in diabetic rats which is in agreement with previous reports (26, 27). This hypoglycemic effect of MT might be due to its role on catecholaminergic responses (28). Also, based on our results, treatment of STZ-induced diabetic rats with three doses of MT increased the gastric antioxidant capacity in a dose-dependent manner. These results are in agreement with previous studies that described that administration of MT improved the oxidative stress induced by diabetes in liver and kidney tissues (29, 30). It has been proven that MT and its metabolites are direct antioxidants and scavengers of free radicals (31). It is likely that MT maintains the mitochondrial function by lowering the level of hydroperoxide, and GSH homeostasis in normal situation and even in oxidative conditions. This means that MT is present in places where free radicals are produced and thus reduces their toxicity. By improving the mitochondrial function and ATP synthesis, the electron transfer rate increases and ROS production is reduced. It has been reported that MT can readily enter the cell and intracellular organelles and protect against intracellular peroxidation damage. MT also detoxifies many free radicals and ROS, including hydroxyl radicals, singlet oxygen and anion peroxyinitrites (32). Other radicals such as peroxy, which cause cell damage, can be neutralized by melatonin (32). Melatonin also directly sweetens H_2O_2 and so prevents the formation of toxic hydroxyl radicals (33), it also stops many complications of diabetes by reducing oxidative stress and prevents individuals from oxidative damage and dyslipidemia (34). In the present study, diabetes was also associated with a significant increase in serum TG, TC and LDL and significant decrease in serum HDL. In many investigations it has been proven that diabetes is associated with major abnormalities in lipid profile including hypertriglyceridemia, hypercholesterolemia, high plasma level of LDL and low plasma level of HDL. Based on previous findings, elevated plasma lipid levels are related to lipid peroxidation in diabetic rats (35). Lipid peroxidation may in turn bring about the oxidative reactions of sugars, that leads to lipid and protein damage in continuous oxidative stress cycles

(36). There are sufficient evidences suggesting that fatty acids, which in normal situations act as energy sources for beta cells, in high concentrations have a toxic effect on these cells and lead to decrease in insulin secretion (34), impaired insulin gene expression (37), and increased beta cell death (38). In the present study, MT significantly improved STZ-induced dyslipidemia. However, the exact mechanisms for this high effectiveness of MT on plasma lipid profile are not fully elucidated, but it seems that potent antioxidant action of MT, as well as its effects in reduction of enteric cholesterol absorption (39, 40), inhibition of cholesterol biosynthesis and LDL accumulation and inhibition of LDL receptors (40) might be involved. In the current study, MT treatment showed no significant effect on serum HDL concentration. However, in MT 20 mg/kg+STZ group, serum level of HDL showed 10% increase compared with diabetic group but this elevation was not significant. The exact mechanism for this finding is not clear, but it might be due to insufficient MT therapeutic dose and short time protocol.

In conclusion, the findings of the present study revealed that melatonin in a dose-dependent manner could improve the gastric oxidative damage and serum dyslipidemia induced in STZ-treated diabetic rats. Further investigations are required to elucidate the mechanism(s) of beneficial actions of melatonin during diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

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