doi: 10.4183/aeb.2015.155

THE EFFECT OF MELATONIN ON GASTRIC PARAMETERS FOLLOWING DIABETES INDUCTION IN MALE RATS

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Abstract

Background. Gastrointestinal complaints are common among diabetic patients. The gastrointestinal tract contains melatonin. The binding sites of melatonin have been identified in all GIT tissues. Melatonin can modify activities of the gut and liver. The aim of this study was to evaluate the possible protective effects of melatonin against gastric motility and secretary responses in Streptozotocin-induced diabetes in rats.

Methods. Streptozotocin was injected intraperitoneally at a single dose of 60 mg/kg for diabetes induction. One week after inducing diabetes, Melatonin (5, 10, 20 mg/kg/day, IP.) was injected for 14 days. Gastric acid and mucus were measured in all animals by chemical methods. Gastric motility was investigated by powerlab system.

Results. Streptozotocin induced a significant increase in blood glucose levels ($p<0.001$) and significant decrease in gastric acid, mucus, motility and body weight in diabetic groups. Treatment of diabetic rats with melatonin significantly reduced blood glucose ($p<0.001$) and increased gastric mucus ($p<0.001$) and motility ($p<0.01$ and $p<0.05$ in groups 4 and 5 respectively) with no effect on body weight and gastric acid concentration.

Conclusion. These data suggested that melatonin treatment has a therapeutic effect on diabetic gastrointestinal disturbances by reduction of serum glucose and increasing gastric motility and gastric mucus levels, but no effect on gastric acid and body weight.

Key words: Melatonin, Motility, Mucus, Acid, Streptozotocin, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The complications of diabetes mellitus include long-standing damage, dysfunction and failure of several organs (1). Diabetes mellitus affects a large number of people of all ages, races and socio-economic classes throughout the world (2). Weight loss is a common complication of diabetes (3). Gastrointestinal complaints are also common among diabetic patients (4). Impaired motor activity of the stomach is one of the most common dysfunctions that causes the condition known as Diabetic gastroparesis (5). Several studies indicate that about 70–75% of diabetic patients have at least one gastrointestinal disorder (4). These disorders in the diabetic patients are attributed to both abnormal gastrointestinal motility and gastrointestinal secretion and absorption (6). Gastrointestinal symptoms like dysphagia, nausea, vomiting, abdominal discomfort, diarrhea, constipation or faecal incontinence are frequently seen in diabetes mellitus (7).

The gastrointestinal tract (GIT) of vertebrate species has melatonin (8). The binding sites of Melatonin have been identified in all GIT tissues, as the lowest number of binding sites are found in oral mucosa and esophagus and the highest number are located in the stomach, duodenum, jejunum, ileum and distal colon (9, 10). The neurohormone melatonin (N-acetyl-5-methoxytryptamine) is produced in the pineal gland from the amino acid tryptophan and is secreted into the circulation in a circadian rhythm (11). It is noteworthy that blood glucose levels also show a circadian rhythm (12) just like melatonin.

Melatonin is also synthesized in the GIT like in the enterochromaffin cells of the intestinal mucosa (13, 14). The melatonin content of the gut is markedly higher than that of the pineal gland (15) and additional melatonin can be accumulated by the GIT from the circulation (16). Higher doses of melatonin have shown

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preventive effects against inflammatory injuries of the GIT tissues (17).

It has been suggested that melatonin may also have an effective role in energy homeostasis by modulating weight and appetite-regulatory hormones such as leptin and ghrelin (18). The evaluation of the relationships between diabetes, glucose metabolism and the effects of melatonin showed that melatonin could effectively normalize the impaired antioxidative status in Streptozotocin (STZ)-induced diabetic rats (19). Also, long-term administration of melatonin in diabetic rats could reduce hyperlipidemia and hyperinsulinemia, and restore unsaturated fatty acids in serum and tissues (20). Melatonin (MLT) was found to modulate various functions of the gut (21, 22) and the liver (23, 24). Although, the presence of this indolamine in the gastrointestinal tract of mammals has been demonstrated (14), its physiological role in the GIT is still not fully clear. It is supposed that melatonin may affect the transit of chyme through the GIT (25).

The aim of this study was to evaluate the effect of melatonin on the gastric motility, gastric acid, gastric mucus, and serum glucose and body weight in diabetic rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (250±20g) were housed in a room at a constant temperature of 25±2°C, with 12-h light/dark cycles, and fed on standard pellet chow and water at liberty. The study was approved by the ethics committee of the School of Medicine, Mashhad University of Medical Sciences.

Study design

All chemical compounds were of analytical type. Rats weighing 250±20 g were divided into the following five groups (n=10):

Group 1: control group (intact group), group 2: untreated diabetic rats, groups 3, 4 and 5: the diabetic rat treated with melatonin at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg respectively.

Diabetes was induced by 60 mg/kg intraperitoneal injection of a single dose of fresh prepared Streptozotocin (Sigma, ALDRICH, SO130-1G) dissolved in normal saline in groups 2, 3, 4 and 5. 10 days after STZ injection, group 2 received normal saline and ethanol % 4, as the solvent of melatonin, intraperitoneally (IP) for 2 weeks every day. Also, 10 days after STZ injection, in melatonin-treated groups (groups 3, 4 and 5), melatonin (MLT) at doses of 5, 10 and 20 mg/kg/day was injected daily for 2 weeks. All injections have been done just before light–off at 6 pm. Seventy-two hours after Streptozotocin (STZ) injection, hyperglycemia was confirmed by measuring blood glucose levels in a tail vein blood sample using a glucometer (Clever check, TD-4230). Rats with blood glucose levels of 300 mg/dL or higher were considered to be diabetic. Since STZ injection to following 10 days, animals were kept untreated for the purpose of revealing complications of diabetes. Melatonin (Sigma, M5250-5G, USA) was dissolved in absolute ethanol and further dilutions were made in saline to a final concentration of 4% ethanol.

All diabetic animals were treated for two weeks and then gastric motility, acid and mucus in addition to serum glucose and body weight were determined. Then, the animals were killed by intra cardiac potassium chloride(KCL) injection and stomach was removed and kept in -80˚ C until mucus assay was done.

Serum glucose and body weight

Body weight and serum glucose were measured four times during the study:1) at the beginning of the study, 2) 72 h after STZ injection, 3) before melatonin injection and 4) at the last day of the experiment.

Rats were fasted overnight and serum glucose levels were detected by a glucometer in the next morning from the tail vein of each rat.

Gastric motility measurement

Rats were deprived of food for 24 hours prior to experiment but had free access to water. Animals were anesthetized with IP injection of Ketamine (40 mg/kg) plus Xylazine (5 mg/kg). The proximal end of duodenum next to pyloric sphincter was incised slightly. A low-capacity latex balloon attached to a catheter was passed into the stomach through the incision and catheter was tied in the pyloric region. Then, 0.5 ml/100 g BW, normal saline was entered into the balloon (26). To record the intragastric pressure, the catheter was connected to a pressure transducer. After waiting for 15 minutes to reach a steady state, gastric motility was measured during 30 minutes (27) by a power lab system (AD Instruments, Australia). Intragastric pressure recording, is an indicator of gastric motility (28). Data transferred to an Excel® file for further processing. Results were expressed as mmHg.

Gastric acid measurement

After measuring intragastric pressure, through an elastic cannula 1 ml physiological saline was entered into the stomach. A period of 30 min was allowed for stabilization; once the gastric acid secretion had been

stable for 30 min, it was considered as basal acid secretion. Throughout the experiment, the gastric secretions were collected in consecutive 15 - min intervals (2 samples). The acid content of each gastric washout sample was measured with a manual titrator pH meter to an end point pH of 7 with 0.01 N NaOH and mean of samples expressed as mEq/ml/15min(29).

Gastric mucus measurement

The gastric mucus was estimated by the method of Corne (30). Briefly, 0.1 g of the glandular part of the stomach was immersed in 2 ml 1% Alcian blue solution for 2 hours. Then tissue was washed twice in 0.5 ml of 25M sucrose solution. After that, the tissue was soaked in 2.5 ml0.5M $MgCl_2$ solution (pH =6) for 2 hours.

The resulting blue solution was mixed with equal volume of diethyl ether and the precipitate was removed by centrifugation at 3600 rpm for 10 min. The supernatant absorbance was measured at 598 nm using a spectrophotometer (Convergys 100, Convergent technologies GmbH and Co. KG, Germany). The results of the mucus level in the gastric mucosa were expressed as mg Alcian blue / g tissue.

Statistics

All values were expressed as mean ± SEM. Statistical analysis was performed by a one way analysis of variance (ANOVA) followed by the Tukey's post-hoc test and paired-samples T-test using SPSSv.16 software. $p<0.05$ was considered as significant.

RESULTS

Body weight

The results of body weight are shown in Table 1. The paired test T test was used for comparison between groups. Seventy-two hours after STZ-injection the body weights were significantly decreased in group 2 ($p<0.01$), group 3 ($p<0.001$), group 4 ($p<0.001$) and group 5 ($p<0.01$) compared to their initial body weight and *vs*. their final body weight ($p<0.001$).

Moreover, body weight in third day after STZ-injection showed a significant decrease in group 3 ($p<0.05$) and groups 4 & 5 ($p<0.01$) compared to group 1. Also, before MLT treatment significant reduction was observed in group 2 ($p<0.01$), group 3, group 4 and group 5 ($p<0.001$) *vs.* group 1. Final body weight of all diabetic groups were significantly decreased vs. group 1 ($p<0.001$).Treatment with melatonin had no effect on weight loss in diabetic animals ($p>0.05$).

Blood glucose levels

Seventy-two hours after STZ injection, serum glucose level was significantly increased in groups 2, 3, 4 and 5 *vs.* group 1 ($p<0.001$). Blood glucose levels before MLT-treatment in second week were significantly decreased in groups 4 and 5 *vs.* their final blood glucose ($p<0.001$) but did not change in group 3 ($p>0.05$) significantly.

Treatment with melatonin for two weeks caused significant decrease in serum glucose levels in

Table 1. The mean of body weight in control rats (group 1), untreated diabetic rats (group 2) and melatonin-treated diabetic rats at doses of 5, 10 and 20 mg/kg (groups 3, 4 & 5 respectively). Statistical analysis was performed by one way ANOVA for comparing among groups and paired samples T test for comparison between groups. Differences with P value <0.05 were considered significant. ***: P<0.001,**: P<0.01 and *: P<0.05 *vs.* group 1

	Group1	Group2	Group3	Group4	Group5
Initial body weight(g)	**247±3.67**	**254.111±4.61**	**252±5.00**	**256.778±4.46**	**238.62±4.16**
Body weight 3days after STZ-injection(g)	**248±3.38**	**235.889±2.31**	**228.636±5.32***	**225.444±3.90****	**225.5±4.62****
Body weight before MLT treatment (second week) (g)	**251.22±3.36**	**225.111±1.89****	**221.909±5.38*****	**211.889±3.32*****	**214.25±4.69*****
The last day body weight (fourth week) (g)	**258.77±2.94**	**207.222±4.83*****	**213.636±6.22*****	**195.889±3.16*****	**208.5±5.04*****

Table 2. The mean of blood glucose levels in control rats (group 1), untreated diabetic rats (group 2) and melatonin-treated diabetic rats at doses of 5, 10 and 20 mg/kg (group 3,4 & 5 respectively). The Tukey's post-hoc test was used to test for differences among means when ANOVA indicated a significant P value (P <0.05). ***: P<0.001 *vs*. group 1, +++: P<0.001 *vs.* group 2

	Group1	Group2	Group3	Group4	Group5
Initial blood glucose (mg/dL)	151.444±2.23	147.889±3.02	150.545±1.79	145.444±2.16	149.125±1.59
Blood glucose 3 days after STZ-injection (mg/dL)	150.66±1.52	331.333±13.50***	369.455±16.45***	344.778±7.63***	365.875±21.99***
Blood glucose before MLT (second week) (mg/dL)	150.00±2.25	347.111±20.54	357.00±20.90	454.333±22.18	377.875±27.53
Final blood glucose (fourth week) (mg/dL)	147.88±1.68	398.111±28.94***	353.273±37.10***	164.444±22.46^{+++}	145±10.36^{+++}

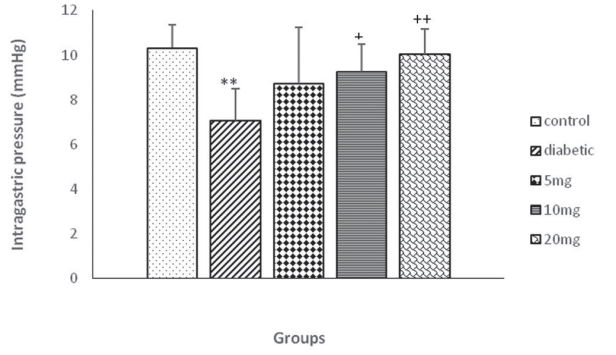

Figure 1. Intragastric pressure (mmHg) in control rats (Group 1), untreated diabetic rats (Group 2) and melatonin-treated diabetic rats at doses of 5, 10 and 20 mg/kg (Groups 3, 4 and 5 respectively). Statistical comparison was performed using one way ANOVA. Differences with P value <0.05 were considered significant. **: P<0.01 *vs.* Group 1, +: P<0.05 and ++: P<0.01 *vs.* Group 2.

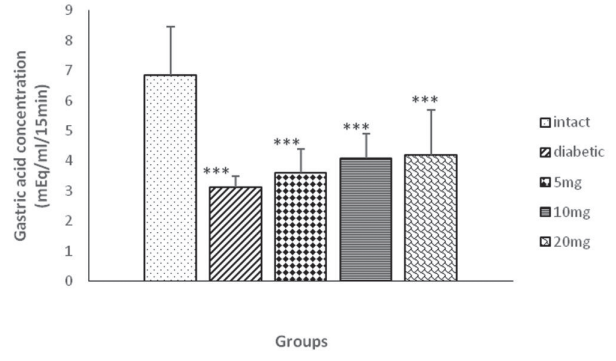

Figure 2. Gastric acid concentration (mEq/mL/15min) in control rats (Group 1), untreated diabetic rats (Group 2) and melatonin-treated diabetic rats at doses of 5, 10 and 20 mg/kg (Groups 3, 4 and 5 respectively). Statistical comparison was performed using one way ANOVA. Differences with P value <0.05 were considered significant. ***: P<0.001 *vs.* Group 1.

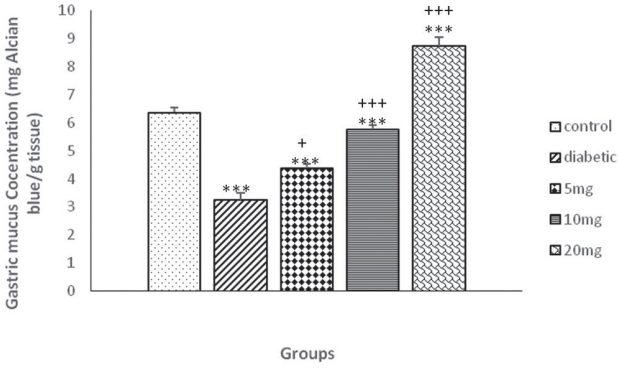

Figure 3. Mucus concentration of control rats (Group 1), untreated diabetic rats (Group 2) and melatonin-treated diabetic rats at doses of 5, 10 and 20 mg/kg (Group 3, 4 and 5 respectively). Data are expressed as mg Alcian blue / g tissue and statistical comparisons were made by one-way analysis of variance followed by the Tukey's post-hoc test. Data were considered statistically significant if p values were lower than 0.05. ***: P<0.001 vs. group 1, +:P<0.05,+++:P<0.001 *vs.* Group 2, °°°: P<0.001 and °°: P<0.01 *vs.* Group 3.

groups 4 and 5 *vs.* group 2 (p<0.001) with no difference compared to group 1 (p>0.05) while significant difference was observed in groups 2 and 3 *vs.* group 1 (p<0.001) (Table 2).

Intragastric pressure

Intragastric pressure in group 2 showed a significant decrease *vs.* group 1 (p<0.01) (Fig. 1). There was a significant increase in intragastric pressure in group 4 (p<0.05) and group 5 (p<0.01) compared to group 2. There was no significant difference between groups 3, 4 and 5 *vs.* group 1.

Gastric acid concentration

As it is shown in Figure 2, basal acid secretion in group 2 (3.1±0.12 mEq/l), group 3 (4.1±0.26 mEq/l), group 4 (3.8±0.27mEq/l) and group 5 (4.1±0.52mEq/l) significantly decreased *vs.* group 1 (6.7±0.53mEq/l) (p<0.001). There was no statistically significant difference in acid levels between group 2 and melatonin treated groups (p>0.05).

Gastric mucus concentration

Gastric mucus concentration is shown in Figure 3 (×1000). As it is shown, Gastric mucus of group 2 (3.25±0.25 mg Alcian blue/g tissue), group 3 (4.37±0.18 mg Alcian blue/g tissue), and group 4 (5.75±0.16 mg Alcian blue/g tissue) were significantly lower than group1(6.37±0.18 mg Alcian blue/g tissue) (p<0.001). But, gastric mucus of group 5 (8.75±0.31 mg Alcian blue/g tissue) showed a significant increase *vs.* group 1 (p<0.001). The Gastric mucus in groups 3 (p<0.05), 4 and 5 showed a significant increase (p<0.001) *vs.* group 2 after melatonin treatment. Gastric mucus levels were significantly higher in groups 4 (p<0.01) and 5 (p<0.001) *vs.* group 3.

DISCUSSION

In the present study, streptozotocin-induced diabetic rats showed an increase in serum glucose and a decrease in body weight that are due to diabetes and its complications. Moreover, gastric motility, gastric mucus and gastric acid secretion decreased significantly after diabetes induction compared to intact animals. Intraperitoneal injection of melatonin (MLT) in diabetic rats showed a hypoglycaemic effect as well as effective role in increasing gastric pressure and mucus levels. However, decreased gastric acid secretion remained almost unchanged after treatment with MLT.

Melatonin treatment decreased serum glucose level in diabetic rats in the present study. This result is in agreement with Gorgun *et al.* (31), Montilla *et*

al. (32) and Anwar *et al.* (33). It has been reported that higher glucose and insulin levels were associated with lower melatonin levels in rats (34, 35). However, several reports have shown that increased melatonin levels are associated with increased blood glucose (36) and decreased insulin secretion (37, 38). Melatonin has been reported to offer protective effect against the development of Alloxan-related diabetes and lipid peroxidation in STZ-diabetic rats (39). A possible mechanism of hypoglycemic effects of melatonin has been reported through its role on catecholaminergic responses (40). In contrast to our study, melatonin did not significantly affect the elevated glucose concentration in streptozotocin-induced diabetic rats (41, 42). There is a controversial discussion on the importance of pineal extracts in glucose metabolism (43, 44). As exogenous melatonin, raises blood glucose levels (45), whereas a decrease in blood glucose (37, 38) and an increase in insulin levels are observed after pinealectomy (46). This controversy may be related to the different way of melatonin administration or different concentrations that have been used.

Our study demonstrated the reduction of body weight in STZ-induced diabetic rats.

Melatonin did not significantly affect weight loss in diabetic and melatonin-treated groups. According to Yavuz, Cam *et al.* body weights were not different in untreated and melatonin-treated diabetic rats (3).

Moreover, we found a characteristic decrease in gastric motility; gastric mucus and acid secretion in STZ- induced diabetic animals. It has been proposed that diabetic gastroparesis is due to neuropathy (47) and this impairment occurs as a result of abnormal metabolism present in long-standing and untreated diabetes (48). Some studies showed that normal levels of glucose can prevent neuropathy and reverse the accumulation of increased glucose at nerve endings, also it reverses the attendant neuropathy (49). However, we have not found any study to show the influence of melatonin on gastric motility, our findings can be attributed to the ameliorative and improving effect of melatonin on nerve circulation (50) or its effective role on removing free radicals (51). In a recent study, melatonin was shown to decrease oxidative stress in streptozotocin-induced diabetic rats (52).

Melatonin has been reported to protect against various types of gastric ulceration through increasing glutathione levels and reducing polymorphonuclear leukocyte infiltration (53). Diabetes has been reported to increase vulnerability of the gastric mucosa to various ulcerogens (54). Achlorhydria and atrophy of gastric mucosa during diabetes may be due to the antibodies affecting parietal cells (55). It has been suggested that long-standing diabetic patients as well as STZ-induced diabetic rats have lower acid levels than normal individuals as a result of vagal neuropathy (56-59). Both the hyperglycemia and hyperglucagonemia in addition to other peptide hormones such as gastric inhibitory peptide can inhibit gastric acid secretion (60) or cause gastric mucosal atrophy due to diabetic autonomic neuropathy (61). It has been indicated that in diabetic rats, the secretory rate of histamine-stimulated gastric H+ was significantly reduced, whereas the mucus secretion was increased (4). In our study, all diabetic rats developed a decrease in acid secretion while Takehara *et al.* study suggested streptozotocin-diabetic conditions impair the HCO_3-secretion in rats (62). No significant increase of acid secretary response was observed in the current study, however, secretion slightly increased by increasing melatonin dose. Beneficial role of melatonin in improving mucus barrier in the present study shows that this neurohormone can provide significant gastroprotective effects in diabetic rats.

In conclusion, Melatonin can modify serum glucose levels without significant effect on weight loss or acid secretion while mucus levels were significantly improved in STZ-induced diabetic rats. Higher doses of melatonin may play a protective role in gastric motility of diabetic rats. Thus, our results suggested that melatonin treatment might have some benefits in controlling diabetic gastrointestinal complications.

Acknowledgment

This study is part of a Msc. Student thesis. The study was financially supported by a research grant from Mashhad University of Medical Sciences.

Conflict of interest

The authors declare no conflict of interests.

Authors' contributions

Dr.Hadjzadeh made substantial contributions to conception and design of the study.

Dr.Keshavarzi participated in analysis and interpretation of data, drafting the article and revising it.

Vajiheh Alikhani took part in writing the article and participated in all experimental steps and animal work.

Sareh Karimi also participated in experimental study and animal work.

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