Protective Effect of Melatonin on Streptozotocin-induced Diabetes in Mice

Bülent Gündüz

The research work was conducted to investigate the effects of melatonin on streptozotocin (STZ)-induced diabetes in mice. Mice were divided into six groups. The first two groups were STZ injected animals. The other four groups were STZ plus melatonin injected. The melatonin doses were 50 or 500 μg kg⁻¹ respectively. Plasma glucose level, body weights and insulines were examined. The plasma glucose levels were significantly higher only in STZ-injected mice. No significant difference was seen in the plasma glucose content of STZ plus melatonin 50 and 500 μg kg⁻¹ groups. Mononuclear cell infiltration of pancreatic islets (insulins) was much more intensive only in STZ injected mice. These results suggested that when melatonin is injected at the same time with STZ, it acts to prevent the development of diabetes in experimental mice.

Key Words: Diabetes, insulines, melatonin, streptozotocin

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Bülent Gündüz: Melatonin and Diabetes in Mice

Introduction
The diabetogenic action of streptozotocin (STZ) results from its highly specific cytotoxic action on the insulin-secreting β cells of the pancreatic islets of Langerhans in experimental animals. The mechanism of STZ-induced diabetes is not quite clear but it has been suggested that oxygen-free radicals, especially hydroxy radicals (OH) may be involved (Baynes, 1986; Oberley, 1986). These radicals are removed by cellular antioxidants. If cellular antioxidants are low, or the production of free radical species exceeds antioxidant defenses, oxidative stress develops (Sies, 1981). Many diabetic complications that are provoked by oxidative stress develop into diabetes mellitus.

Oxidative stress in diabetes mellitus is increased by two mechanisms, one, free radicals produced during increased glucose auto-oxidation, second, the consumption in regeneration of natural antioxidants. In the past several years it has been recognized that the decarboxylation of melatonin is the most efficient free radical scavenger and antioxidant (et al., 1991; Tan et al., 1989; Ebeli et al., 2000). Melatonin mediates the physiological and endocrinological processes particularly reproduction. Melatonin level is high during the night in both nocturnal and diurnal animals. It has been shown to influence in terms of antioxidative activity. Because of high liposolubility, it readily scavenges the most toxic free radical and detoxifies the peroxynitrite anion, nitric oxide, singlet oxygen, and the peroxy radical. It may also stimulate the several antioxidative enzymes including superoxide dismutase and glutathione peroxidase. All these actions may all contribute to melatonin's ability to reduce oxidative damage (Staskie et al., 1986; Longoni et al., 1989; Peter et al., 1997; Peter et al., 1998).

Melatonin's role on the glucose metabolism in diabetes is controversial. In rodents, the role of melatonin in glucose metabolism has been investigated using either exogenous melatonin administration or a chronic suppression of endogenous melatonin synthesis by the pineal gland through surgical pinealectomy. It has been reported that melatonin treatment increases glycemia (Ortega-Corona et al., 1989), whereas other studies showed no effect (Bailey et al., 1974; Frenkel and Brandberg, 1984; Saljo Devi et al., 2000) or even a decrease (Kuzu, 1988). In rabbits, melatonin induces a decrease in plasma glucose concentration in basal conditions, whereas it induces an enhancement of the hyperglycemia after a glucose load (Dhara, 1993). More recently, administration of melatonin prior to STZ treatment decreased serum glucose level in mice (Abdel-Wahab and Abd-Allah, 2000).

Materials and Methods
Male BALB/c mice, 8-12 months old, weighing 30-35 g were purchased and housed 5 per cage and kept under a 12 h light-dark cycle lights off at 2000 h at 21 ± 1°C with free access to food and tap water. Body weights were measured at the beginning of the experiment. 48 mice were divided into six groups of treatment (Table 1). Diabetes was induced by STZ injections (Abdel-Wahab and Abd-Allah, 2000), given daily for 5 consecutive days with the dose of 40 mg kg⁻¹ freshly dissolved in citrate buffer and melatonin was injected subcutaneously (Melamit et al., 2000) at a dose of either 60 (Groups 3 and 4) or 5000 µg kg⁻¹ (Groups 5 and 6) respectively, 15 minutes before the injection of STZ from day 1 to day 5, and continued daily for either 10 or 20 days. Control animals in Group-1 and Group-2 were injected with citrate buffer alone after the completion of STZ injections. Melatonin and STZ injections were made at mid-day.

Mice were killed by decapitation and blood was drawn on heparinized tubes. Plasma was separated by centrifugation of blood at 1000 rpm for 30 min. An aliquot of fresh plasma (10 µL) was used to assay glucose by a glucose oxidase, enzymatic colorimetric method (Glucose Assay Kit, Sigma, USA). After decapitating, the pancreas glands were removed, fixed in 10% formalin solution and embedded in paraffin. 10 µm sections were stained with hematoxylin and eosin. Insulins was described by heavy monoclonal cell infiltration into majority of the islets. Two independent examiners being unaware of the origin of the samples evaluated the pancreas slides. Body weights of live mice were taken at the beginning and at the end of experiment.

Comparison was performed by one-way analysis of variance (ANOVA, SAS Inst. Ver. 6.06) followed by Newman-Keuls multiple comparisons. Values are reported as Mean ± SEM. The level of significance was < 0.05.

Results
Initial and final body weights are shown in Fig. 1. A marked decrease in final body weights (p < 0.01) was noticed after 20 days injection of STZ only in Group-2. Plasma glucose levels are shown in Fig. 2. Plasma glucose level from mice in Group-1 and Group-2 was more than 400 mg dl⁻¹. STZ administration in these two groups caused severe hyperglycemia (p < 0.001). Plasma glucose values higher than 200 mg dl⁻¹ were regarded as diabetic. Mice receiving STZ plus melatonin either 50 or 5000 µg kg⁻¹ doses showed a significant decrease in plasma glucose by day 11 (p < 0.05 after the last injection of STZ) or by day 21 compared to normal STZ injected control. There was no significant change in plasma glucose levels between mice receiving daily injection of STZ plus melatonin at a dose of 50 and of STZ plus melatonin at a dose of 5000 µg kg⁻¹ respectively for either 10 or for 20 days. Analysis of pancreatic tissue was reported only in terms of insulin. In the STZ injected groups (Group-1, Fig. 3A and Group-2, Fig. 4A), the appearance of insulin was higher compared to STZ plus melatonin (either 50 and 5000 µg kg⁻¹, Fig. 3B or 5000 µg kg⁻¹, Fig. 4B) injected groups.

Fig. 1: Effects of streptozotocin (STZ) and melatonin treatments on body weights in mice. Group-1 represents 5-day-STZ injection and decapitation at day 11, Group-2 represents 5-day-STZ injection and decapitation at day 21, Group-3 represents 5-day-STZ and 10-day-Melatonin (50 µg) injections, Group-4 represents 5-day-STZ and 20-day-Melatonin (50 µg) injections, Group-5 represents 5-day-STZ and 50-day-Melatonin (500 µg) injections and finally Group-6 represents 5-day-STZ and 10-day-melatonin (500 µg) injections. Asterisk (*) indicates significant difference compared to all other mice of the experiment. Open bars indicate initial body weights and the cross-hatched bars indicate final body weights. Data are presented as the Mean ± SEM for each group.
### Table 1: Daily streptozotocin (STZ) and melatonin (Mel) injections. STZ was given 5 consecutive days in all groups. Group 1 and Group 2 were treated with STZ only and in Group 3 after STZ injections, saline buffer (b) injections continued until Day 10. Animals were sacrificed at day 11. In Group 3 after STZ injections, saline buffer (b) injections continued until Day 50 and animals were sacrificed at Day 51. In Group 2 and Group 4 animals were treated with 800 μg melatonin (Mel) for 10 days (Group 3) and for 20 days (Group 4). In group 5 and 6 animals were treated with 600 μg Mel for 10 days (Group 5) and for 20 days (Group 6). Streptozotocin (STZ) 40 mg kg⁻¹, Melatonin (Mel) 80 or 600 μg kg⁻¹, 0.9% saline buffer (b) 4.5 ml. (Table)

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### Fig. 2: Plasma glucose concentrations in mice treated with injections of either streptozotocin (STZ) or melatonin. STZ was given for 5 consecutive days. Values are Mean ± SEM for each group. Similar letters indicate statistically similar.

### Discussion

This study demonstrates that melatonin treatment protects against STZ-induced beta cell toxicity in mice. When given at a dose of either 80 or 600 μg kg⁻¹ respectively, daily, melatonin resulted in complete protection from diabetes for at least 21 days. STZ-induced diabetes has been widely used animal model and is characterized by insulin deficiency and many other chemical and pathological features shared by human type I diabetes (Sheriff, 1990). Considering the acute effect, the injection of both STZ and melatonin did not lead to changes in glucose metabolism and pancreas histology. It should be noted that injection of melatonin was performed at the time of day-night cycle when melatonin levels are at their low level. This protective effect of melatonin may or may not be depend on a modulation of glucose metabolism. These results...
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4A STZ+ MelDay: 21 600ua

Fig. 4 Pancreatic tissue of STZ-injected mice (Group 2). Heavy mononuclear cell infiltration is seen around the islets (4A, x200). Melatonin administration (4B, x200) reduces the infiltration.

References


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