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Protective Effect of Melatonin on Streptozotocin-induced Diabetes in Mice

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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 500μg kg^{-1} respectively. Plasma glucose level, body weights and insulates 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 500μg kg^{-1} groups. Mononuclear cell infiltration of pancreatic islets (insulates) 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, insulates, melatonin, streptozotocin

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Introduction

The diabetic 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 hydroxyl radicals (·OH) may be involved (Baynes, 1991; Oberley, 1988). 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, 1991). Many diabetic complications that were provoked by oxidative stress develop into diabetes mellitus.

Oxidative stress in diabetes mellitus is increased by two mechanisms, one; free radicals are produced during increased glucose auto-oxidation, second; the corruption in regeneration of natural antioxidants. In the past several years it has been recognized that pineal hormone melatonin is the most efficient free radical scavenger and antioxidant (*et al*., 1991; Tan *et al*., 1993; Ebelt *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 several functions in terms of antioxidatic ability. Because of high lipid-solubility, 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 (Stasica *et al*., 1998; Longoni *et al*., 1998; Reiter, 1997; Reiter *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*., 1991), whereas other studies showed no effect (Bailey *et al*., 1974; Frankel and Strandberg, 1991; Sailoja Devi *et al*., 2000) or even a decrease (Iizuka, 1996). 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 (Dhar *et al*., 1983). 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 8 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 40mg kg^{-1} freshly dissolved in citrate buffer and melatonin was injected subcutaneously (Maritim *et al*., 2000) at a dose of either 50 (Groups 3 and 4) or 500Fg 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 6000 rpm for 30 min. An aliquot of fresh plasma (10 Fl) was used to assay glucose by a glucose oxidase, enzymatic calorimetric method (Glucose Assay Kit, Sigma, USA). After decapitating, the pancreatic glands were removed, fixed in 10% formalin solution and embedded in paraffin. 10 Fm sections were stained with hematoxylin and eosin. Insulates was described by heavy mononuclear cell infiltration into a majority of islets. Two independent examiners being unaware of the origin of the sections 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 400mg dl^{-1}.STZ administration in these two groups caused severe hyperglycemia ($p < 0.001$). Plasma glucose values higher than 200mg dl^{-1} were regarded as diabetic. Mice receiving STZ plus melatonin either 50 or 500Fg kg^{-1} doses showed a significant decrease in plasma glucose by day 11 (i.e. 6 days after the last injection of STZ) or by day 21 compared to normal STZ injected control. There was no significant change in plasma glucose level between mice receiving daily injection of STZ plus melatonin at a dose of 50 and of STZ plus melatonin at a dose of 500Fg kg^{-1} respectively either for 10 days or for 20 days. Analysis of pancreatic tissue is reported only in terms of insulates. In the STZ injected groups (Group-1, Fig. 3A and Group-2, Fig. 4A), the appearance of insulates was higher compare to STZ plus Mel (either 50 Fg kg^{-1}, Fig. 3B or 500Fg kg^{-1} Fig. 4B) injected groups.

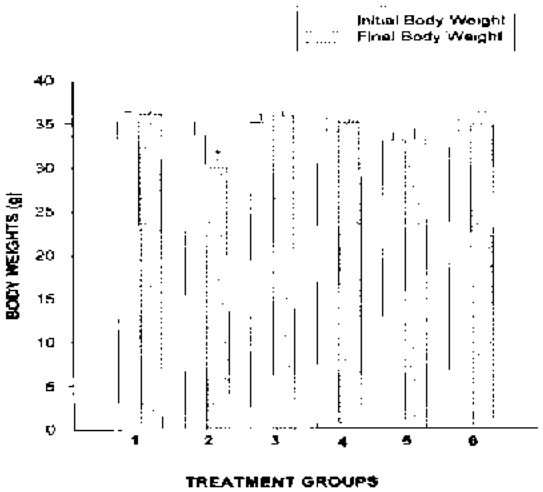


Fig. 1: Effects of streptozotocin (STZ) and melatonin treatments on body weights in mice. Group-1 represents 5-day-STZ injection and decapitation at day 11, Group-2 represents 5-day-STZ injection and decapitation at day 21, Group-3 represents 5-day-STZ and 10-day-Melatonin (50 μg) injections, Group-4 represents 5-day-STZ and 20-day-melatonin (50 μg) injections, Group-5 represents 5-day-STZ and 10-day-melatonin (500 μg) injections and finally Group-6 represents 5-day-STZ and 20-day-melatonin (500 μg) injections. Asterisk (*) indicates significant difference ($p < 0.01$) from all other groups 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-1 after STZ injections, citrate buffer (cb) injections continued until Day 10, animals were sacrificed at day 11. In Group-2 after STZ injections cb injections continued until day 20 and animals were sacrificed at day 21. In Group 3 and 4 animals were treated with 50 μg melatonin (Mel) for 10 days (Group-3) and for 20 days (Group-4). In group 5 and 6 animals were treated with 500 μg Mel for 10 days (Group-5) and for 20 days (Group-6). Streptozotocin (STZ): 40 mg kg^{-1}, Melatonin (Mel): 50 or 500 μg kg^{-1}, cb: Citrate buffer (pH: 4.5), X: Decapitation

Days	Group-1	Group2	Group-3 (50μg)	Group-4 (500μg)	Group-5 (500 μg)	Group-6 (500μg)
1	STZ	STZ	STZ + Mel	STZ + Mel	STZ + Mel	STZ + Mel
2	STZ	STZ	STZ + Mel	STZ + Mel	STZ + Mel	STZ + Mel
3	STZ	STZ	STZ + Mel	STZ + Mel	STZ + Mel	STZ + Mel
4	STZ	STZ	STZ + Mel	STZ + Mel	STZ + Mel	STZ + Mel
5	STZ	STZ	STZ + Mel	STZ + Mel	STZ + Mel	STZ + Mel
6	cb	cb	Mel	Mel	Mel	Mel
7	cb	cb	Mel	Mel	Mel	Mel
8	cb	cb	Mel	Mel	Mel	Mel
9	cb	cb	Mel	Mel	Mel	Mel
10	cb	cb	Mel	Mel	Mel	Mel
11	X	cb	X	Mel	X	Mel
12		cb		Mel		Mel
13		cb		Mel		Mel
14		cb		Mel		Mel
15		cb		Mel		Mel
16		cb		Mel		Mel
17		cb		Mel		Mel
18		cb		Mel		Mel
19		cb		Mel		Mel
20		cb		Mel		Mel
21		X		X		X

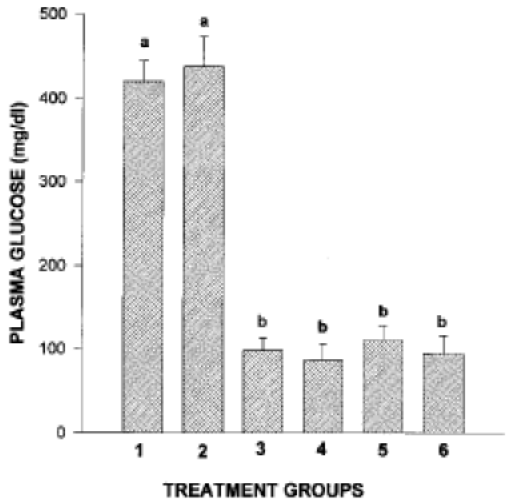
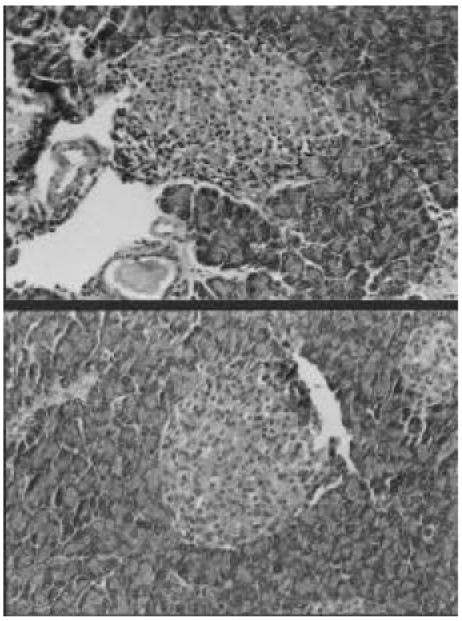


Fig. 2: Plasma glucose concentrations in mice treated with injections of either streptozotocin (STZ) or melatonin. STZ was given for 5 consecutive days. Values are Mean ± SEM for each group. Similar letters indicate statistically similar.

3A: STZ Day-11



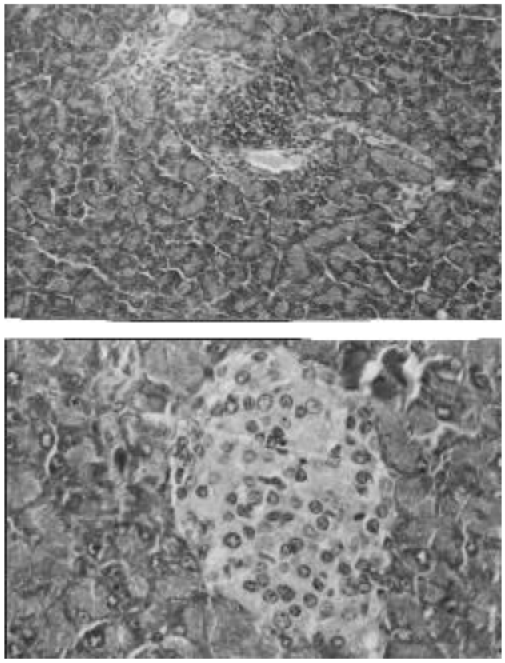
3B: STZ + Mel 50μg) Day-11

Fig. 3: Protective effect of melatonin on STZ-induced histological changes in the Langerhans islets of mice. 3A (x200): Photography of the pancreas of a control mouse (Group-1). In this photography mice were treated with STZ only. 3B (x200): photography of the pancreas of a STZ plus melatonin (50 μg) treated mouse (Group-3).

Discussion

This study demonstrates that melatonin treatment protects against STZ-induced beta cell toxicity in mice. When given at a dose of either 50 or 500Fg 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 otherbio chemical and pathological features shared by human type I diabetes (Sheriff, 1996). Considering the acute effect, the injection of both STZ and melatonin did 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

4A STZ Day-21



4B STZ+ Mel Day-21 500μg

Fig. 4: Pancreatic tissue of STZ injected mouse (Group-2). Heavy mononuclear cell infiltration is seen around the Langerhans islets (4A, x200). Photography of STZ plus melatonin (500 μg; Group-6) injected mice (4B, x400).

do not in fact allow any definitive conclusion.

The interpretation of the decrease in plasma glucose values after melatonin treatment should also take into account the fact that STZ-induced diabetes is partly immune-mediated. Melatonin protect β cell not only from STZ but also from autoimmune process by inducing a β cell rest as insulin does in other model (Atkinson *et al*., 1990). This possibility was investigated by evaluating the pancreatic state and the appearance of insulates by histological analysis in this investigation. Apart from the possibility that autoimmunity is affected by the melatonin treatment, one has to considered that melatonin might alter the insulin sensitivity in the STZ-induced mice.

Considering the pineal hormone melatonin's general effect, it is possible to formulate a hypothesis. The pineal gland hormone has previously been demonstrated to be an effective free radical scavenger and antioxidant and might therefore, be potentially capable of preventing the development of diabetes induced by STZ. Tan *et al*. (1993) reported that melatonin neutralizes many toxic radical such as (OH). In case of autoimmune diabetes, oxygen free radicals, released after lymphocytic infiltration of the pancreas, are known to be the mediators of pancreatic β cell damage (Oberley, 1988). The protective effects of anti-oxidants on diabetes has been reported by various scientists (Mamelak, 1989; Lazarus and Shapiro, 1973; Yang and Cherian, 1994; Uchigata *et al*., 1983; Pierrefiche *et al*., 1991) but melatonin deserves further studies with a focus on its antioxidant potential and mechanism of action.

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