MELATONIN AMELIORATES HYPERINSULINEMIA, GLUCOSE INTOLERANCE AND INSULIN RESISTANCE IN STZ-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Objective: The aim of the present study was to explore the effect of melatonin on hyperinsulinemia, glucose intolerance and insulin resistance in type 2 diabetic rats.

Methods: Type 2 diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg), 15 min after the i.p. administration of nicotinamide (120 mg/kg). Hyperglycemia was confirmed by the elevated glucose level (>200 mg/dl) in the blood determined at 72 h after STZ-nicotinamide injection. After the confirmation of diabetes melatonin (8 mg/kg & 16 mg/kg) was administered orally once a day for 15 days.

Results: There was “a” significant increase in blood glucose, glycosylated hemoglobin (HbA C) and serum insulin levels observed in type 2 diabetic control rats. Treatment with melatonin reduced the elevated levels of blood glucose, HbA C and insulin in type 2 diabetic rats. An oral glucose tolerance test (OGTT) was also performed in the same groups, in which we found a significant improvement in glucose tolerance in the rats treated with melatonin. There was significant rise in homeostasis model assessment of insulin resistance (HOMA-R) in type 2 diabetic control rats whereas melatonin treatment significantly prevented the rise in HOMA-R in type 2 diabetic treated rats.

Conclusion: The present study clearly suggests that melatonin is pharmacologically effective in ameliorating hyperinsulinemia, glucose intolerance and insulin resistance in type 2 diabetic rats.

Keywords: Melatonin, Streptozotocin, Hyperglycemia, Hyperinsulinemia, Insulin sensitivity.

INTRODUCTION

Type 2 diabetes mellitus (DM) is a significant growing health problem in developed and developing countries and is the most common serious metabolic disorder. It is characterized by hyperglycemia that results from an absolute or relative deficiency in insulin secretion [1] on one side, and insulin resistance on the other [2,3]. The progression of type 2 DM begins with an impairment of glucose tolerance [4] and is often associated with a state of insulin resistance, which means insulin that is secreted by the β-cells and bound to liver, muscle and fat cells, is sub normally efficacious in carrying out its metabolic actions [5].

Insulin resistance is the core pathophysiological feature of a number of other health disorders, including obesity, glucose intolerance, dyslipidemia and hypertension clustering in the so-called metabolic syndrome (also commonly referred to as syndrome X). Management of type 2 DM without any side effect is still a challenge to the medical system. Previous studies from our laboratory have demonstrated that treatment of Withania somnifera and Rhus coriaria on hyperinsulinemia, glucose intolerance and insulin sensitivity in STZ-induced type 2 diabetic rats [3, 6].

Melatonin is one of the tryptophan derivatives that is mainly synthesized in the pineal gland. In addition to physiologic functions such as controlling day-night rhythm, inducing sleep, seasonal regulation of reproduction and increasing immune system activity, melatonin has been known as a potent scavenger of hydroxyl and peroxyl radicals and regulates activity of antioxidant enzymes [7, 8]. Previously it has been shown to exhibit neuroprotection under a variety of circumstances [9, 10]. This endogenously produced antioxidant is capable of scavenging both reactive oxygen and reactive nitrogen species [11]. Melatonin has been shown in several studies to protect against nuclear DNA damage, cataract formation and seizures caused by free radicals [12-14]. Previous report also showed that melatonin protects the kidney and pancreatic tissue of STZ-induced rats [15, 16]. Hence, the present study was carried out whether hyperinsulinemia, glucose intolerance and insulin resistance in STZ-nicotinamide induced type 2 diabetic rats could be prevented and/or reversed with melatonin treatment. The ultimate goal of the study was to illustrate the therapeutic potential of melatonin for the treatment of type 2 diabetes mellitus.

MATERIALS AND METHODS

Experimental Animals

Healthy albino Wistar rats were kept for breeding. The animals were maintained under controlled condition of illumination (12 hr light/12 hr darkness) and temperature 20-25°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet and water ad libitum throughout the experimental period. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India and conducted as per the CPCSEA guidelines.

Drugs and Chemicals

Streptozotocin (STZ) and melatonin were purchased from Sigma, Chemicals Company, USA. The enzyme-linked immunosorbent assay (ELISA) kit for insulin assay was purchased from Merodia (Uppsala, Sweden). Glucose kit was purchased from Span diagnostics, India. All the other biochemicals and chemicals used for the experiment were of analytical grade.

Induction of Diabetes

Type 2 diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg), 15 min after the i.p. administration of nicotinamide (120 mg/kg) [17]. Hyperglycemia was confirmed by the elevated glucose level in the blood determined at 72 h after STZ-nicotinamide injection. Rats with fasting blood glucose levels of 200 mg/dl or higher were considered to be diabetic and were used in this experiment. After the confirmation of diabetes melatonin (8 mg/kg & 16 mg/kg) was administered orally once a day for 15 days.

Experimental design

The rats were divided into four groups comprising of six animals in each group as follows:

Group I: Normal control rats, received saline (1 ml/kg, p.o) orally daily for 15 days.

Group II: Type 2 diabetic rats received STZ (60 mg/kg, i.p) in normal buffer (pH- 4.5), 15 min after nicotinamide administration (120 mg/kg, i.p).

Group III: Type 2 diabetic treated rats received melatonin (8 mg/kg, i.p) in 0.3% tween 80 daily for 15 days.

Group IV: Type 2 diabetic treated rats received melatonin (16 mg/kg, i.p) in 0.3% tween 80 daily for 15 days. On 16th day of the treatment blood samples were collected for biochemical estimation.

**Determination of Blood Glucose**

Blood glucose level was estimated by glucose oxidase-peroxidase method [18] using a commercial diagnostic kit.

**Determination of Glycosylated Hemoglobin (HbA1c) level**

Glycosylated haemoglobin level was estimated by Bannon method [19] using a commercial diagnostic kit.

**Determination of Insulin Level**

Plasma insulin level was estimated quantitatively by ELISA method of Morgan and Lazarow [20]. For this purpose insulin ELISA kit was used.

**Determination of OGTT**

OGTT was measured according to the method of Pari and Saravanan [21]. Glucose solution (2 g/kg) was given to overnight fasted rats. Blood samples were taken at 0, 15, 30, 60 and 120 min after glucose administration. All the blood samples were collected for glucose estimation.

**Determination of Insulin Resistance**

Insulin resistance was determined using homeostasis model assessment of insulin resistance (HOMA-R) using the following formula:

$$\text{HOMA-R} = \frac{\text{Fasting insulin (µU/mL)} \times \text{fasting blood glucose (mmol/L)}}{22.5}$$

**Statistical Analysis**

Data were expressed as the mean ± standard error (SE) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis.

The Tukey-Kramer post hoc test was applied to identify significance among groups. P < 0.05 was considered to be statistically significant.

**RESULTS**

**Effect of melatonin on hyperglycaemia in type 2 diabetic rats**

Table 1 shows the effect of melatonin on the blood glucose levels. Significant (P<0.001) increase in blood glucose level was observed in type 2 diabetic control rats when compared with normal control rats. Oral administration of melatonin at two doses (8 mg/kg and 16 mg/kg) reduced the blood glucose level significantly (P < 0.001) when compared with type 2 diabetic control rats.

**Effect of melatonin on HbA1c in type 2 diabetic rats**

Table 1 shows the effect of melatonin on glycosylated hemoglobin levels. Significant (P < 0.001) increase in HbA1c levels were observed in type 2 diabetic control rats when compared with normal control rats. Oral administration of melatonin at two doses (8 mg/kg and 16 mg/kg) decreased the HbA1c level significantly (P < 0.001 - P < 0.001) in a dose-dependent manner when compared with type 2 diabetic control rats.

**Table 1: Effect of melatonin on blood glucose and glycosylated haemoglobin levels in type 2 diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose (mg/dl)</th>
<th>Glycosylated Haemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>15 min</td>
</tr>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>89.16±5.21</td>
<td>6.08±0.265</td>
</tr>
<tr>
<td>II</td>
<td>Type 2 diabetic control(STZ 100 mg/kg, intraperitoneally)</td>
<td>265.02±9.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.33±0.322&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Type 2 diabetic + Melatonin (8 mg/kg, i.p)</td>
<td>165.89±6.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.09±0.219&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Type 2 diabetic + Melatonin (16 mg/kg, i.p)</td>
<td>134.51±6.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.53±0.129&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± S.E; n=6 in each group. *P < 0.001 compared with the corresponding value for normal control rats (group I). **P < 0.001, ***P < 0.01, ****P < 0.05 compared with the corresponding value for type 2 diabetic control rats (group II).

**Effect of melatonin on OGTT in type 2 diabetic rats**

Table 2 shows the blood glucose levels of normal control, type 2 diabetic control and type 2 diabetic treated rats after oral administration of glucose (2 g/kg). In type 2 diabetic control rats the peak increase in blood glucose levels were observed after 1 h. The blood glucose levels remained high over next one hour. Melatonin treated rats showed significant (P<0.001) decrease in blood glucose levels at 1 and 2 h when compared with type 2 diabetic control rats.

**Table 2: Effect of melatonin on oral glucose tolerance test (OGTT) in type 2 diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose (mg/dl)</th>
<th>Glycosylated Haemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>15 min</td>
</tr>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>98.92±1.67</td>
<td>119.20±2.05</td>
</tr>
<tr>
<td>II</td>
<td>Type 2 diabetic control(STZ 100 mg/kg, intraperitoneally)</td>
<td>267.56±7.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>283.51±7.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Type 2 diabetic + Melatonin (8 mg/kg, i.p)</td>
<td>187.32±1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>199.07±1.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Type 2 diabetic + Melatonin (16 mg/kg, i.p)</td>
<td>139.71±1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>148.31±2.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± S.E; n=6 in each group. *P < 0.001 compared with the corresponding value for normal control rats (group I). **P < 0.001 compared with the corresponding value for type 2 diabetic control rats (group II).

**Effect of melatonin on insulin levels in type 2 diabetic rats**

Table 3 shows the effect of melatonin on insulin levels. Hyperinsulinemia was significantly (P < 0.001) observed in type 2 diabetic control rats when compared with normal control rats. Melatonin treatment significantly (P < 0.001) reduced the elevated level of insulin when compared with type 2 diabetic control rats.

**Effect of melatonin on insulin resistance in type 2 diabetic rats**

Table 3 shows the level of HOMA-R, an index of insulin resistance. Type 2 diabetic control rats showed significant (P < 0.001) increase in insulin resistance compared with normal control rats. Oral administration of melatonin at two doses (8 mg/kg and 16 mg/kg) reduced the insulin resistance significantly (P < 0.001) when compared with type 2 diabetic control rats.
in HOMA-R level when compared with normal control rats. Treatment with melatonin significantly \((P < 0.001)\) decreased HOMA-R level when compared with type 2 diabetic control rats.

**DISCUSSION**

Type 2 DM is a chronic metabolic disorder characterized by hyperglycemia due to decreased secretion of insulin and/or insulin sensitivity [23]. Treatment that is inadequate or instituted too late predisposes the affected individual not only to the basic metabolic disturbances but also to a number of serious complications of diabetes. STZ-nicotinamide is frequently used to induce type 2 DM in experimental animals [24, 25].

Although, it is well accepted that the cytotoxicity produced by STZ depends on DNA alkylation and subsequent activation of poly ADP-ribose synthetase causes rapid and lethal depletion of NAD in pancreatic islets [26, 27]. Several lines of evidence indicate that the free radicals may play an essential role in the mechanism of β-cell damage and diabeticogenic effect of STZ [28]. Treatment with nicotinamide provides protection to the pancreatic β-cell against free radicals and oxidative stress which has been expressed to cytotoxic compound such as streptozotocin (STZ).

Type 2 diabetic control rats showed significant elevation in fasting blood glucose, fasting serum insulin and HOMA-R values indicating development of insulin resistance. These findings are consistent with our previous reports [3, 6]. Treatment with melatonin to type 2 diabetic rats reduced the elevated blood glucose levels. In diabetes, there is an increased glycosylation of a number of proteins including hemoglobin and β-crystalline of lens [29]. Measurement of glycosylated hemoglobin (HbA\(_c\)) has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes [30]. HbA\(_c\) levels increased in type 2 diabetic control rats when compared with normal control rats. Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with protein glycation [31]. Melatonin, whose antioxidant properties are well documented, is attracting increased attention in recent years and is known to reduce oxidative damage [32]. Administration of melatonin to type 2 diabetic rats reduced the glycosylation of hemoglobin by virtue of its free radical scavenging property and thus decreased the levels of HbA\(_c\). A decrease in blood glucose levels might also contribute to decreased levels of glycated haemoglobin in melatonin treated type 2 diabetic rats.

**Table 3: Effect of melatonin on insulin level and HOMA-R in type 2 diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Insulin Level (µU/ml)</th>
<th>HOMA-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>16.15±0.245</td>
<td>3.56±0.179</td>
</tr>
<tr>
<td>II</td>
<td>Type 2 diabetic control (STZ 100 mg/kg, intraperitoneally)</td>
<td>29.09±0.329&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.03±0.431&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Type 2 diabetic + Melatonin (8 mg/kg, i.p)</td>
<td>24.51±0.222&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.03±0.338&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Type 2 diabetic + Melatonin (16 mg/kg, i.p)</td>
<td>19.57±0.200&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.49±0.284&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± S.E; n=6 in each group. \(P < 0.001\) compared with the corresponding value for type 2 diabetic control rats (group II).

Hyperinsulinemia appears to be a compensatory mechanism that responds to increased levels of circulating glucose and is often associated with the progression to insulin resistance [33]. The β-cells normally compensate insulin resistance by secreting more amounts of insulin to maintain glucose homeostasis. Bonora et al. [34] has reported that hyperinsulinemia is associated with decreased hepatic insulin clearance and hypersecretion of β-cells in mild glucose intolerance obese subjects.

Results of the present study clearly showed that hyperinsulinemia (as evident by increased serum insulin levels) was seen in type 2 diabetic control rats. Therefore, the hyperinsulinemia in type 2 diabetic rats could be either due to decreased hepatic clearance of insulin or by down-regulation of insulin receptors and desensitizing postreceptor pathways [35], resulting in decreased insulin binding and degradation.

Despite high insulin levels (hyperinsulinemia), the glucose levels were greater in type 2 diabetic control rats than type 2 diabetic treated rats. However, melatonin treatment was found to be effective in reducing insulin levels of type 2 diabetic rats, thereby preventing hyperinsulinemia. It seems that melatonin exerts antihyperglycemic effect by attenuating hyperinsulinemia.

An insulin-resistance state is a key phase of metabolic syndrome, constituting the major risk factor for the development of glucose intolerance and diabetes mellitus [36]. Thus interventions to decrease insulin resistance may postpone the development of diabetic complications. When animals were subjected to OGTT, increased blood glucose levels were found with increase in time and were maintained until 2 h in type 2 diabetic rats. In the present investigation the rate of glucose disposal was found to be significantly decreased in type 2 diabetic control rats.

Treatment with melatonin significantly improved glucose tolerance, as indicated by reduction in peak blood glucose levels at 1 and 2 h in type 2 diabetic treated rats during OGTT. Melatonin might enhance glucose utilization by peripheral tissues and increasing the glycogen stores in the liver due to restoration of delayed insulin response because it significantly decreased the blood glucose levels in glucose loaded rats. Our results showed that melatonin decreased blood glucose levels, prevented hyperinsulinemia and improved glucose tolerance in type 2 diabetic rats.

These results suggest that melatonin can ameliorate insulin resistance. Thus HOMA-R level was determined to check insulin resistance. The results obtained clearly showed that melatonin treatment significantly prevented the rise in HOMA-R in type 2 diabetic treated rats.

**CONCLUSION**

These findings suggest that melatonin is pharmacologically effective in ameliorating hyperinsulinemia, glucose intolerance and insulin resistance in type 2 diabetic rats. If these results are extrapolated in humans then melatonin at proper dose might be useful in the treatment and/or prevention of type 2 diabetes mellitus.

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