Effect of chronic treatment with losartan on streptozocin induced diabetic rats

B Murali & R K Goyal*
Department of Pharmacology, L.M. College of Pharmacy, Ahmedabad, 380 009, India
Received 30 November 2000; revised 16 August 2001

Treatment of rats with streptozocin (STZ, 45mg/kg, i.v., single dose) produced cardinal symptoms of diabetes mellitus including hyperglycemia, hypoinsulinemia and increase in blood pressure. Treatment with losartan—an angiotensin (AT1) receptor antagonist, 2 mg/kg, po for 6 weeks decreased the blood glucose levels by 16.5%. There was 190% increase in AUCglucose and 59.4% decrease in AUCinsulin in STZ-diabetic rats as compared to control rats. Treatment with losartan caused slight decrease in AUCglucose and slight increase in AUCinsulin. There was no significant difference in insulin sensitivity (KITT) index of STZ-diabetic group as compared to control. Losartan treatment failed to alter these levels significantly. Serum cholesterol and creatinine levels were found to be increased significantly in STZ-diabetic rats. Treatment with losartan significantly prevented the rise in cholesterol and creatinine levels by 20.1 and 81% respectively. The results suggest that losartan produces some beneficial effects in STZ-diabetic rats.

Diabetes mellitus and hypertension are common chronic conditions that frequently co-exist. Approximately 80% of diabetic patients are hypertensive whereas 5-25% of hypertensive individuals are diabetic. Arterial hypertension is twice as common in both Type I (insulin dependent diabetes mellitus or IDDM) and Type II (non-insulin dependent or NIDDM) diabetes as compared to general population. Both hypertension and diabetes mellitus are multifaceted and dynamic expressions of pathological disequilibria, which are closely related and even intermingled by common factors such as obesity, hyperinsulinemia, micro- and macro-vascular diseases and cardiac risk factors. Hypertension is more prevalent among diabetics than non-diabetic subjects and is reported to aggravate the cardiovascular complications of diabetes. It contributes to increased morbidity and mortality, due to coronary artery disease and end-stage renal disease (ESRD) in diabetic patients.

During studies on the effects of antihypertensive agents on cardiovascular and metabolic complications in various models of diabetes and hypertension, it was observed that while all antihypertensive agents studied could effectively lower blood pressure, there are differences with respect to the effectiveness of different classes of drugs on cardiovascular, metabolic and functional alterations induced by diabetes and/or hypertension. Angiotensin converting enzyme inhibitors (ACEI) were found to be metabolically neutral and could correct some of the untoward metabolic actions of diuretics such as hypokalaemia and hypercholesterolaemia. Therefore, ACEI appear to be more effective in preventing long-term complications of hypertension when associated with diabetes mellitus including cardiovascular and renal complications. ACEI are more effective in controlling blood pressure in diabetic conditions. Their use is limited because of disadvantages like unproductive cough and angioedema. These difficulties have been overcome by the introduction of specific angiotensin receptor antagonist.

Losartan, a prototype selective non-peptide AT1 receptor antagonist, is potent and orally active agent. By inhibiting the binding of angiotensin II at AT1 receptors, losartan and its active metabolite (E 3174) block the vasconstrictor and aldosterone secreting effects of angiotensin II, regardless of the source of secretion of angiotensin II. The present investigation has been undertaken to study the effects of chronic treatment of angiotensin receptor (AT1) antagonist, losartan on STZ-induced diabetic rats.

Materials and Methods

Induction of diabetes and experimental protocol—Female albino rats of Wistar strain weighing 200-250 g were used. Diabetes was induced by a single tail vein injection of STZ (45 mg/kg, iv) dissolved in saline. Control group received normal saline. Animals were checked for the intensity of glucosuria using diastix (Bayer Diagnostics India Ltd, Vadodara, India). Animals showing glucosuria (>2%) 48 hr after injection of STZ were considered as diabetic. These animals were randomly divided into 4 sub-groups:-
control, control treated with losartan, diabetic control and diabetic treated with losartan. Losartan was administered as a single dose of 2mg/kg/day, po for 6 weeks. All the groups were maintained for 6 weeks with food and water given ad libitum throughout the period. They were observed for water intake, food intake, changes in body weight and mortality during the period of study.

**Blood sample collection and analysis**—At the end of 6th week, the animals were fasted for 5 hr. The blood samples were collected, allowed to clot and the serum was separated by centrifugation. The serum samples were analysed for glucose, cholesterol and creatinine levels using kits (Glaxo, Span and Bayer Diagnostics, India). Serum insulin was measured by radioimmunoassay method using kit obtained from Board of Radioactivity and Isotope Technology (BRIT) Mumbai, India.

**Measurement of blood pressure**—After completion of 6 weeks treatment, blood pressure was recorded by indirect tail cuff method using Harvard blood pressure device (Kent, UK). Rat was placed in the restrainer and allowed to calm down for 5 min and a cuff with a photodetector fitted over its tail. The cuff was inflated to record the blood pressure on the chart.

**Oral glucose tolerance test (OGTT)**—Rats were fasted for at least 5 hr after the last meal. They were given 1.5 g/kg glucose (po) and blood samples were collected at the intervals of 0, 10, 20, 30, 60 and 120 min. Glucose and insulin were estimated as mentioned above. Results are expressed as integrated area under the curve (AUC) for glucose and insulin by the trapezoid rule [AUC = (C1+C2/2 x t2 – t1)] and changes in glucose and insulin concentrations during OGTT are expressed as AUCglucose (mg/dl/min) and AUCinsulin (µU/ml/min) respectively.

**Insulin tolerance test (KITT)**—Insulin Tolerance Test is used to assess peripheral insulin resistance. This test measures insulin sensitivity using KITT as an index of insulin mediated glucose metabolism. Rats were fasted for 5 hr before giving insulin challenge. Neutral insulin injection (Actrapid Novo, Ahmedabad, India) was diluted with 0.9% saline to get the final concentration of 0.2 U/0.1 ml. This insulin was injected in the dose of 0.2 U/100 g body weight by slow iv injection through tail vein. Blood samples were collected at 0 min (before giving insulin injection), and then at 10, 20, 30 and 60 min after giving insulin injection. Serum was subjected to glucose estimation. KITT was determined from the slope of a linear portion of the regression line of natural logarithm of glucose versus time. Using a formula, calculation of KITT was carried out as follows.

$$K_{ITT} = \frac{0.693 \times 100}{t_{1/2}}$$

where $t_{1/2}$ represents the half-life of plasma glucose decay. The half-life of plasma glucose was obtained by plotting plasma glucose concentrations and time on semilogarithmic graph paper.

**Statistical analysis**—The data were analyzed statistically using analysis of variance followed by Tukey's test. Value of $P<0.05$ was considered significant.

**Results**

**General features of animals**—Injection of STZ produced glucosuria (>2%) in all the animals. No detectable glucose was present in the urine of control animals. There was a significant loss of body weight, polyphagia and polydipsia in diabetic animals (Table 1). However, there was a normal weight gain in control and losartan treated control rats. Treatment with losartan produced slight but significant decrease in loss of body weight, polydipsia and polyphagia in diabetic animals. The mean blood pressure was significantly increased after 6 weeks study period in diabetics as compared to controls and losartan treatment prevented hypertension in diabetic animals (Table 1).

**Biochemical parameters**—There was a significant increase in blood glucose levels in STZ-diabetic rats. Losartan treatment significantly decreased STZ-induced increase in glucose levels by 16.5% (Table 2). STZ-diabetic rats had significant hypoinsulinemia, which was not altered by losartan treatment. There was a significant increase (190%) in AUCglucose and decrease (59.4%) in AUCinsulin in STZ-diabetic rats as compared to control rats. However, losartan treatment produced slight decrease in AUCglucose and increase in AUCinsulin. There was no alteration in KITT value of STZ-diabetic animals as compared to control or losartan treated diabetic animals (Table 2). Serum cholesterol and creatinine were found to be increased significantly in the STZ-diabetic rats. Treatment with losartan significantly prevented the STZ-induced increase in cholesterol by (20.1%) and creatinine levels by (81%).
Discussion

Intravenous injection of STZ produced various cardinal symptoms of diabetes such as, hyperglycemia, hypoinsulinemia, loss of body weight, polyphagia, polyurea and polydipsia. These findings are consistent with earlier findings. Losartan treatment significantly prevented the loss in body weight in diabetic animals. There was normal gain in body weight in non-diabetic animals. Treatment with losartan prevented polydipsia and polyphagia in diabetic rats.

Serum glucose levels in STZ-diabetic rats were significantly higher than the control. Injection of STZ produced a decrease in insulin levels and rise in blood pressure in these animals. This is similar to that of earlier reports. Treatment with losartan significantly prevented STZ-induced hypertension in diabetic rats without altering insulin levels. Inspite of insulin deficiency, losartan significantly decreased glucose levels in diabetic rats. It is possible that inhibition of angiotensin II receptors produces decrease in glucose levels by improvement in insulin sensitivity. Losartan has been reported to improve insulin sensitivity, in clinical settings.

In the present study, there was a significant increase in AUC$_{glucose}$ and a significant decrease in AUC$_{insulin}$ values in STZ-diabetic rats as compared to control rats. Treatment with losartan failed to alter these levels significantly. It is possible that STZ-diabetic rats are not insulin resistant and hence losartan failed to alter these values. However, there was no significant difference between K$_{ITT}$ value of control, diabetic control and losartan treated diabetic group.

Earlier studies have shown that diabetes mellitus is associated with changes in lipid metabolism. Rats injected with STZ increased plasma levels of triglycerides, cholesterol, free fatty acids and phospholipids. In the present study significant elevation in cholesterol levels was observed in diabetic animals. Hypoinsulinemic condition, may be responsible for the elevation of cholesterol levels in diabetic animals because insulin has an inhibitory

Table 1—Effect of losartan on general parameters of experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Food intake (g/rat/day)</th>
<th>Water intake (ml/rat/day)</th>
<th>Mean blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6)</td>
<td>288.1 ± 10.3</td>
<td>15 ± 7.0</td>
<td>27.8 ± 6.5</td>
<td>114 ± 6.43</td>
</tr>
<tr>
<td>Control treated (7)</td>
<td>270.0 ± 11.3</td>
<td>16.25 ± 4.5</td>
<td>26.3 ± 7.0</td>
<td>109 ± 1.53</td>
</tr>
<tr>
<td>Dia.Control (6)</td>
<td>152.5 ± 13.4*</td>
<td>60.0 ± 7.8*</td>
<td>155.0 ± 8.6*</td>
<td>145.7 ± 8.2*</td>
</tr>
<tr>
<td>Dia.Treated (7)</td>
<td>188.2 ± 8.6*b</td>
<td>40.0 ± 7.1*b</td>
<td>75.0 ± 5.3*b</td>
<td>117.4 ± 5.8*b</td>
</tr>
</tbody>
</table>

*Significantly different from Control (P < 0.05)

Table 2—Effect of losartan on biochemical parameters of experimental animals after 6 weeks treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>S. Glucose (mg/dl)</th>
<th>S. Insulin (µL)</th>
<th>AUC$_{glucose}$ (mg/dl/min.)</th>
<th>AUC$_{insulin}$ (µu/m/min.)</th>
<th>K$_{ITT}$ (min.$^{-1}$)</th>
<th>S. Cholesterol (mg/dl)</th>
<th>S. Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con. (6)</td>
<td>108.32 ± 3.35</td>
<td>33.2 ± 3.24</td>
<td>16135.67 ± 923.88</td>
<td>5981.6 ± 608.67</td>
<td>8.41 ± 0.14</td>
<td>75.22 ± 0.19</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>Con. Treated (7)</td>
<td>104.47 ± 4.4</td>
<td>34.7 ± 2.8</td>
<td>11710.1 ± 866.08</td>
<td>5613.34 ± 139.29</td>
<td>9.36 ± 0.9</td>
<td>84.55 ± 3.23</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>Dia. Control (6)</td>
<td>404.9 ± 12.8*</td>
<td>12.3 ± 1.04*</td>
<td>46852.2 ± 1720.9*</td>
<td>2428.75 ± 923.57*</td>
<td>12.3 ± 1.04*</td>
<td>134.82 ± 12.35*</td>
<td>2.58 ± 0.1*</td>
</tr>
<tr>
<td>Dia. Treated (7)</td>
<td>338.16 ± 19.2*</td>
<td>14.3 ± 1.2*</td>
<td>44927.6 ± 5552.9*</td>
<td>2995.0 ± 194.46*</td>
<td>107.66 ± 2.15*</td>
<td>107.66 ± 2.15*</td>
<td>0.49 ± 0.03*</td>
</tr>
</tbody>
</table>

*Significantly different from Control (P < 0.05)

Significantly different from Diabetic Control (P < 0.05)
action on HMG-CoA reductase, a key enzyme that acts as a rate limiting in the metabolism of cholesterol rich LDL particles. Losartan treatment significantly reduced cholesterol levels in diabetic animals without altering insulin levels. The decrease in cholesterol levels in diabetic rats may be due to improvement in insulin sensitivity by losartan. Similar decrease has also been reported in normotensive offspring of hypertensive parents. Rise in serum creatinine has been reported in patients with diabetes, thereby suggesting that diabetic condition causes renal dysfunction. In the present investigation also we found significant elevation in creatinine in diabetic animals. Treatment with losartan significantly prevented the elevated serum creatinine level. To conclude, the present data suggest that, treatment with losartan on glycemic control.

References
14 Kawashima H, Chronic hypertension induced by STZ in rats, Naunyn Schiemedberg Arch Pharmacol, 305 (1978) 125.