ATTENUATION OF BLOOD SUGAR LOWERING EFFECT OF GLIMEPIRIDE BY CONCURRENT USE OF AMLODIPINE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Summary

In the present study, an attempt has been made to evaluate possible interaction between glimepiride and amlodipine when administered concurrently in both normoglycemic and diabetic rats. In normoglycemic rats blood glucose level after single dose administration was measured. Diabetes was induced by streptozotocin (60 mg/kg, i.p.). All the diabetic rats were administered with glimepiride (1mg/kg p.o.) alone and in combination with amlodipine (5mg/kg p.o.) once daily for 3 weeks. Lipid profiles like cholesterol, triglyceride levels and oxidation parameters like SGOT, SGPT were evaluated along with glucose level in diabetic rats on day 0 and on day 22. The glucose was estimated by the help of glucometer. Blood was collected from retro-orbital sinus for the estimation of other parameters. In both normoglycemic and diabetic rats, the combination is less effective than the glimepiride alone in reducing the sugar, triglyceride and cholesterol level. Glimepiride alone as well as the combination significantly reduced SGOT and SGPT level. So the study suggests that use of amlodipine and glimepiride combination should not be preferred in patients suffering from both diabetes and hypertension.

Key words: Combination therapy, Amlodipine, Glimepiride, Streptozotocin, blood sugar.
Introduction

Diabetes is a group of disorders characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. Chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of organs like eye, kidney, nerve, heart and blood vessels. Hypertension is an extremely common co-morbid condition in diabetes, affecting nearly 20-60% of patients with diabetes, depending on obesity, ethnicity and age. In type-2 diabetes, hypertension is often present as part of the metabolic syndrome of insulin resistance. Type-1 diabetes may reflect onset of diabetic nephropathy. In U.K. Prospective Diabetes Study (UKPDS) epidemiological study, each 10 mm Hg decrease in mean BP was associated with reductions in risk of 12% for any complication related to diabetes, 15% for death related to diabetes.

Glimepiride is a newer, novel second generation sulphonylurea. It increases insulin secretion by stimulating beta cells and also has significant extrapancreatic activity. Glimepiride binds to its receptor 2.5 to 3 times faster and dissociates from its binding site 8 to 9 times greater than glibenclamide. Glimepiride exhibits more pronounced insulin independent blood glucose decreasing activity as compared to glibenclamide.

The calcium channel blockers can alter blood glucose level as calcium ions have a role in insulin secretion from pancreatic beta cells by the process of exocytosis. Oxidative stress alters insulin secretion resulting in diabetes and hyperglycemia. The calcium channel blocker like amlodipine is reported to have antioxidant effect. So the aim of the present study is to find out the influence of amlodipine on the glucose lowering effect of glimepiride in normoglycemic and STZ induced diabetic rats.

Materials and Methods

Animals

Albino rats of either sex (150-250gm) were procured from the animal house of Gayatri College of Pharmacy, Sambalpur, Orissa, India. Albino rats of either sex weighing 150-200 gm were used. Lighting was artificial in animal room. 12-hour light and 12-hour dark sequence was maintained. Conventional laboratory diet was used as feed with drinking water ad libitum. All the protocols were approved by the Institutional Animal Ethical Committee (1339 / ac / 10 / CPCSEA).
Drugs and Chemicals

Estimation of glucose was done by glucometer (Onetouch Horizon, Johnson & Johnson ltd). SGOT, SGPT, Cholesterol and triglyceride were determined by standard methods using assay kits (Span Diagnostics ltd, India). Amlodipine and Glimeperide were procured as gift samples from Aristo Pharma Ltd, India. Streptozotocin (Sigma Aldrich) was used to induce diabetes in rats.

Study on normoglycemic rats

The evaluation of glucose lowering effect of Glimepiride (1mg/kg p.o.) and its combination with amlodipine (2.5 mg/kg and 5mg/kg p.o.) on normoglycemic rats was a single dose study. Rats were divided into 4 groups (n=6) out of which one group served as control. The animals were fasted for 12 hours before the test. Blood samples were collected from tail vein at 0, 1, 2, 4, 6 and 24 hrs after drug administration for estimation of glucose level.

Induction of experimental diabetes

Rats were made diabetic with single dose of Streptozotocin (60 mg/kg, i.p. 7). The STZ was dissolved in a citrate buffer solution (pH 4.5). After 72 hrs, animals with fasting glucose level >200 mg/dl were considered as diabetic and were employed in the study.

Experimental design

The diabetic rats were divided into 3 groups (n=6). In addition to these groups another group (n=6) of normoglycaemic rats served as control. Both the control and the diabetic control groups were administered with the vehicle. Drugs were administered once daily for 3 weeks in the form of suspension using tween 80 as the suspending agent.

Group-1: Control group, receive solvent and tween 80.
Group-2: Diabetic control, receive solvent and tween 80.
Group-3: Diabetic test, treated with Glimepiride (1mg/kg).
Group-4: Diabetic test, treated with Glimepiride (1mg/kg) and amlodipine (5mg/kg).
The blood samples were collected on end of every week from the tail for estimation of glucose. At the end of 3 weeks, the blood samples were collected by retro orbital puncture, blood was allowed to clot, centrifuged and serum separated. The biochemical parameters like cholesterol, triglyceride, SGOT and SGPT were estimated by colorimeter.

**Statistical Analysis**

The data obtained in the present study are expressed as Mean ± SEM and were analyzed by one way ANOVA followed by Dunnet’s t-test. The values were considered statistically significant when p<0.05.

**Results**

As shown in Table 1, in normoglycemic rats, Glimepiride (1mg/kg p.o.) significantly reduces blood glucose level (hypoglycemia) of normoglycemic rats after 2hr, 4hr and 6hr of its administration. The glucose level after 24 hours is normal. However, there is no significant reduction in blood sugar when amlodipine (2.5 mg/kg and 5mg/kg p.o.) is co-administered with Glimepiride.

Streptozotocin significantly increased the sugar level. As shown in Table 2 Glimepiride (1mg/kg p.o.) significantly reduced the blood sugar level on day 8 and 15 in streptozotocin induced diabetic rats and was found to be normal on day 22. But when amlodipine (5 mg/kg p.o.) was administered in combination with Glimepiride (1 mg/kg p.o.), the blood sugar level was significantly increased as compared to glimepiride alone. So amlodipine blocks the blood sugar lowering effect of Glimepiride.
Table 1. Effect of Glimepiride (1 mg/kg p.o.) alone and in combination with amlodipine (2.5 mg/kg and 5 mg/kg p.o.) on blood glucose (mean ± SD) in normoglycemic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and Dose (mg/kg)</th>
<th>Initial Glucose Level (mg/dl)</th>
<th>Final Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>91.17 ±9.54</td>
<td>87.50 ±8.26</td>
</tr>
<tr>
<td>II</td>
<td>Glimepiride (1)</td>
<td>94.33 ±9.31</td>
<td>70.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±7.00*</td>
</tr>
<tr>
<td>III</td>
<td>Glimepiride (1) + Amlodipine (2.5)</td>
<td>93.33 ±10.29</td>
<td>72.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±7.37*</td>
</tr>
<tr>
<td>IV</td>
<td>Glimepiride (1) + Amlodipine (5)</td>
<td>86.52 ±9.59</td>
<td>74.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±9.14*</td>
</tr>
</tbody>
</table>

One way ANOVA followed by Dunnet’s t-test. n=6. *p<0.05
Group II, III and IV are compared with Group I.
Table 2. Effect of Glimepiride (1 mg/kg p.o.) alone and in combination with amlodipine (5 mg/kg p.o.) on blood glucose (mean ± SD) in streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and Dose (mg/kg)</th>
<th>Initial Glucose Level (mg/dl)</th>
<th>Final Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 day</td>
</tr>
<tr>
<td>I</td>
<td>Control (Vehicle)</td>
<td>87.00±9.01</td>
<td>85.33±9.73</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control (Vehicle)</td>
<td>340.33±44.12*</td>
<td>342.00±45.40*</td>
</tr>
<tr>
<td>III</td>
<td>Glimepiride (1)</td>
<td>362.05±46.43</td>
<td>96.67±28.08*</td>
</tr>
<tr>
<td>IV</td>
<td>Glimepiride (1) + Amlodipine (5)</td>
<td>334.17±40.71</td>
<td>309.50±42.82*</td>
</tr>
</tbody>
</table>

One way ANOVA followed by Dunnet’s t-test. n=6. *p<0.05
Group II is compared with Group I, Group III is compared with Group II and Group IV is compared with Group III.
Marked increase in serum triglyceride and cholesterol observed in untreated diabetic rats which is in line with the previous findings. Under normal circumstances, insulin activates the enzyme lipoprotein lipase and hydrolyses the triglycerides of circulation. Insulin deficiency results in failure to activate the enzyme thereby causing hypertriglyceridemia. As shown in Table 3, Glimepiride (1 mg/kg p.o.) alone decreases the triglyceride and cholesterol level significantly which was reversed by concurrent use of amlodipine (5 mg/kg p.o.). Elevation of SGOT and SGPT like biomarker enzymes is observed in diabetic control rats which indicate the cardiac damage and hepatic damage. The hepatic damage is restored and elevated transaminase was significantly reduced by oral antidiabetic drugs like Glimepiride. The diabetic complications like gluconeogenesis and ketogenesis may be due to elevated transaminase activity. Glimepiride (1 mg/kg p.o.) significantly reduced the SGOT and SGPT level in streptozotocin induced diabetic rats. However, this effect of glimepiride on SGOT was significantly reversed on co-administration with amlodipine (5 mg/kg p.o.) and effect on SGPT was unaltered.

Discussion

The present study was done to verify possible interaction between Glimepiride and amlodipine when administered concurrently in normoglycemic and streptozotocin induced diabetic rats. Glucose level was considered as the primary parameter of the study. The biochemical parameters like SGOT, SGPT, cholesterol and triglyceride were also estimated as these parameters also change in diabetic rats.

Glimepiride is a newer, novel second generation sulphfonylurea. It increases insulin secretion by stimulating beta cells of islet of langerhans of pancreas and also has significant extrapancreatic activity. It stimulates glucose transport and nonoxidative glucose metabolism in adipose tissue and muscle cells. It reduces glucose level of STZ induced diabetic rats. It has been observed in animal studies that platelet inhibitory effect of glimepiride is more pronounced and hence it may have a preventive effect in the development of microvascular complications.
Table 3. Effect of Glimepiride (1 mg/kg p.o.) alone and in combination with amlodipine (5 mg/kg p.o.) on triglyceride, cholesterol, SGOT and SGPT (mean ± SD) in streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and Dose (mg/kg)</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>SGOT</th>
<th>SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (Vehicle)</td>
<td>97.50±11.38</td>
<td>98.50±11.11</td>
<td>59.67±9.71</td>
<td>50.50±8.87</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control (Vehicle)</td>
<td>154.33±15.3*</td>
<td>140.33±8.82*</td>
<td>288.17±20.69*</td>
<td>161.4±17.94*</td>
</tr>
<tr>
<td>III</td>
<td>Glimepiride (1)</td>
<td>64.67±9.07*</td>
<td>64.33±8.33*</td>
<td>145.00±10.49*</td>
<td>56.33±10.31*</td>
</tr>
<tr>
<td>IV</td>
<td>Glimepiride(1) + Amlodipine (5)</td>
<td>130.50±10.89*</td>
<td>96.50±13.00*</td>
<td>65.50±13.40*</td>
<td>63.50±11.18</td>
</tr>
</tbody>
</table>

One way ANOVA followed by Dunnet’s t-test. n=6. *p<0.05

Group II is compared with Group I, Group III is compared with Group II and Group IV is compared with Group III.
The experiment was done taking amlodipine in the dose 5 mg/kg and used continuously for 3 weeks. The result obtained from the experiment on normoglycemic as well as diabetic rats don’t support any significant role of amlodipine in reducing blood glucose level in streptozotocin induced diabetic animals. Moreover inhibits the effect of Glimepiride when used in combination. Glimepiride (1 mg/kg p.o.) alone decreases the triglyceride and cholesterol level significantly which was reversed by concurrent use of amlodipine (5 mg/kg p.o.). Glimepiride (1 mg/kg p.o.) significantly reduced the SGOT and SGPT level in streptozotocin induced diabetic rats. However, this effect of glimepiride on SGOT was significantly reversed on co-administration with amlodipine (5 mg/kg p.o.) and effect on SGPT was unaltered.

Calcium is necessary for the secretion of insulin\(^4\). So calcium channel blockers are likely interact with the effect of antidiabetic drugs. As there is a possibility of combined use of these two drugs in patients suffering from diabetes associated with hypertension, the present study is designed to find out the safety of the combination.

Calcium channel blockers are widely used for the treatment of hypertension\(^13\). The calcium channel blocker like amlodipine reduced the blood glucose reduction by second generation sulfonylurea like gliclazide in alloxan induced diabetic animals\(^14\). The daily insulin requirement increased by 30% following nifedipine administrations which suggest that altered glycemic control associated with nifedipine was mediated by suppressed beta cell function\(^15\). It is reported that calcium channel blockers produce hyperglycemia in rats by inhibiting insulin release and reduced uptake of glucose by peripheral tissue\(^16\).

Calcium channel blockers appear to exert their cytoprotective effects through several mechanisms. These may involve blockade of calcium channels, reduction of oxidative stress, antagonism at inflammatory mediator receptor sites and interaction at other intracellular sites. Studies relating to the liver are few but suggest that calcium channel blockers may have a role to play in limiting hepatocellular damage, especially those arising from exposure to a variety of toxic agents\(^17\). Calcium channel blocker like deltiazem has nephroprotective effect in alloxan induced beagle dogs\(^18\).

The influence of calcium channel blockers on glucose is controversial. Amlodipine; a dihydropyridine derivative has protective role against mitochondrial injury in ischemia and reperfusion injury in rats which may be due to its antioxidant property\(^19\). Amlodipine was also proved to have high antioxidant effect.
among the calcium channel blockers\textsuperscript{20}. Chronic treatment of amlodipine (5 mg/kg, p.o.) for 6 week in streptozotocin (i.v.) diabetic and spontaneous hypertensive rats causes prevention of streptozotocin induced hyperglycemia in both STZ-diabetic wistar and SH (spontaneously hypertensive) rats. The insulin level were decreased in non-diabetic treated wistar rats but were unaltered in non-diabetic SH and diabetic wistar and SH rats. Also there was significant reduction in cholesterol level in diabetic wistar and SH rats\textsuperscript{21}. Chronic treatment of amlodipine (5mg/kg) for 6 week in noninsulin dependent diabetic rats decrease in insulin release and reduction of glucose level occurs significantly which indicates amlodipine increases insulin sensitivity\textsuperscript{22}. But the present study suggests that amlodipine blocks the action of glimepiride.

**Conclusion**

The present study suggests use of amlodipine and glimepiride combination should not be preferred to the patients those are suffering from diabetes co-existing hypertension. Amlodipine being a calcium channel blocker, it blocks the insulin release from the pancreas. The triglyceride as well as cholesterol level also don't decrease which may be due to deficiency of insulin release. Only the SGOT level decreases more by the combination which may be due to antioxidant effect of amlodipine. Though amlodipine increases the insulin sensitivity in NIDDM rats, it is not effective in reducing the glucose level in STZ induced diabetic rats when used in combination with glimepiride than the effect of glimepiride alone.

**References**

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