EFFECTS OF ENALAPRIL ON BLOOD GLUCOSE LEVEL AND INTERACTION WITH THE ORAL ANTI-DIABETIC DRUGS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

AIM: To evaluate the effect of Enalapril on blood glucose level in normal rats and interaction with the oral anti-diabetic drugs in Alloxan-induced diabetic rats.

METHOD: The rats were classified into nine groups (n = 6). The Group I – II were normal and Group III – IX were diabetic. The experimental diabetes in rats was induced by intra-peritoneal injection of Alloxan monohydrate (135 mg/kg body weight). The Enalapril at the dose of 3.2 mg/kg body weight was administered to the normal rats to find-out the effect on blood glucose level. In diabetic groups, the Enalapril was given along with three oral anti-diabetic drugs to know the any alteration on blood glucose level. All drugs administered orally once a day for 13 days and at the end of the experimentation oral glucose tolerance test (OGTT) and blood glucose estimation was done.

RESULTS: It was observed that in normal rats the Enalapril slightly reduced the blood glucose level at all time points which was not significant (P>0.05) but in Alloxan-induced diabetic rats Enalapril exhibited the anti-hyperglycemic activity at all time points in highly significant manner (P<0.001).

CONCLUSION: The present study has showed that Enalapril has significant anti-hyperglycemic activities which enhance the effect of oral anti-diabetic drugs in diabetic animal. It is therefore advisable to prescribe and use of ACE inhibitors with anti-diabetic drugs carefully with necessary dose adjustment to avoid adverse hypoglycemic episodes in diabetic individuals.

KEYWORDS: Anti-hyperglycemic effect; Enalapril; Alloxan monohydrate; Oral Anti-diabetic drugs; Wistar rats

INTRODUCTION

Diabetes Mellitus (DM) is a major threat to global public health that is rapidly getting worse and highest impact in on adult of working age in developing countries1. There are an estimated 246 million people with diabetes in the world, of whom about 80% live in developing countries2. It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels3. Diabetic nephropathy (DN) is the leading cause of end-stage renal failure in Western countries and carries a 20- to 40-fold increased risk for cardiovascular (CV) mortality. In the past 2 decades, there has been a continual increase in the incidence of ESRD among patients with diabetes, predominantly those with type 2 diabetes4. Diabetic nephropathy typically present as microalbuminuria before progressing to macroalbuminuria, a decrease in creatinine clearance and finally renal failure5. The Angiotensin converting enzyme (ACE) inhibitor are the drugs of choice in diabetic nephropathy as they control both systemic hypertension and intra-glomerular hypertension by inhibiting the action of angiotensin II on the systemic vasculature and renal efferent arterioles so ACE inhibitors have been recommended as the treatment of choice for all patients with diabetic nephropathy regardless of diabetes type6,7.

The anti-diabetic drug therapy requires a continued supervision for optimal blood sugar level in patients because strict blood sugar regulation can prevent many of the complications of diabetes like retinopathy, nephropathy, neuropathy and vascular derangements. Avoidance of hypoglycemia is also important because that is also dangerous and can lead to convulsion, coma and death. It is therefore necessary to know interaction of various pharmacological agents with anti-diabetic agents because knowledge of such an interaction enables the physicians to avoid or minimize such an interaction by adjustment of doses and schedule of drug administration or by using an alternative drugs. Various drugs have known interaction with anti-diabetic agents. Some drugs causing hypoglycemia, some hyperglycemia and altering the response of diabetic patients to their existing therapeutic regimens.

Diabetes and hypertension are both independent risk factors for cardiovascular disease and the risk is even greater when nephropathy is present. Cardiovascular causes account for more than half of the mortality associated with nephropathy8. ACE inhibitors are first line drugs for hypertension in diabetes and till date actual impact of ACE inhibitors on blood glucose is not clear, that is why, the present study was planned to see interactions of ACE inhibitor Enalapril with various oral hypoglycemic agents in experimental animal.

MATERIALS AND METHODS

Animals

The study was conducted on healthy albino wistar rats of either sex weighing 150-250 gm. The animals were made available in the Central animal house, GSV Medical College, Kanpur. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light/dark cycle, at room temperature 25±3°C and 35-60% humidity), fed in standard pelleted diet and water ad libitum. The study was approved by the Institutional Animal Ethics Committee, GSV Medical College, Kanpur. The animal care and handling was done as per the guidelines set by CPCSEA and the Indian National Science Academy, New Delhi, India.

Induction of Experimental Diabetes:

Diabetes was induced in albino rats of either sex by a single intra-peritoneal injection of aqueous Alloxan monohydrate (135 mg/kg body weight) (Sigma Chemical Co. USA)9. Blood samples were collected before and after the administration of Alloxan to know the status of diabetes. After two days, diabetes was confirmed by testing blood glucose level using glucometer and they were further maintained for four days for well establishment of diabetes. The
animals with blood glucose level more than 200 mg/dl (moderate diabetes) were selected for the experiment.

**Drugs and chemical agents**

Alloxan- Alloxan monohydrate (dose-135 mg/kg body weight/rat), ACE inhibitor- Enalapril was (dose of 3.6 mg/kg body weight/day/rat). Oral anti-diabetics drugs- (a) Biguanides- Metformin (225 mg/kg body weight/day/rat). (b) Second generation Sulfonylureas- Glimepiride (22 mg/kg body weight/day/rat). (c) Peroxisome Proliferator-Activated Receptor-gamma (PPAR-gamma) agonists-Pioglitazone (4 mg/kg body weight/day/rat).

**Experimental Design**

The rats were classified into nine groups (n=6). The Group I-II were normal and Group III-IX were diabetic. The Animals in Group I (NC) were administered with 1 ml sterile water and served as normal control. Rats of Group II (NE) were treated with drug Enalapril. Rats of diabetic Group III (DC) administered with 1 ml sterile water served as diabetic control. Rats of diabetic Group IV (DM) were treated with drug Metformin, Group V (DG) with drug Glimepiride and Group VI (DP) with drug Pioglitazone. The oral anti-diabetic drugs treated groups (Group IV-VI) served as positive control for the rest of the groups. Rats of diabetic Group VII (D+M+E) were treated with drugs Enalapril and Metformin both, Group VIII (D+G) with drugs Enalapril and Glimepiride both and Group IX (D+P+E) with drugs Enalapril and Pioglitazone both. The animals of all groups were received the doses orally for 13 consecutive days and at the end of the experimentation an Oral Glucose Tolerance Test (OGTT) and blood glucose estimation was done in all groups.

**Oral Glucose Tolerance Test**

After overnight fasting, 0 min blood samples (0.2 ml) were taken from the all groups by orbital sinus puncture. Glucose solution (2 g/kg of 25% w/v) was administered orally in OGTT. Three more samples were taken at 30 min, 60 min and 120 min after glucose administration.

**Blood Glucose Estimation**

Blood samples (0.2 ml) were collected in fluoride vials, from orbital sinus puncture with capillary tube under aseptic conditions. Blood samples were then centrifuged at 3000 rpm for 10 min. The clear supernatant serum was taken for estimation of blood glucose level.

**Statistical analysis**

Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by independent student t-test. Results were considered to be significant when P values were less than 0.05 (P<0.05).

**DISCUSSION**

Our study has evaluated the significant interaction of Enalapril with the different groups of Oral hypoglycemic drugs in Alloxan induced diabetic rats. Alloxan is a well-known diabetogenic agent widely used to chemically induce Type 2 diabetes in experimental animals. Alloxan and its reduction product dialuric acid establish a redox cycle with formation of superoxide radicals, hydrogen peroxide and lastly highly reactive hydroxyl radicals. The action of reactive oxygen species with simultaneous massive increase in intracellular calcium concentration causes rapid destruction of β-cells. The Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content. The Moderate diabetic animals are recommended for use in testing drugs for use in Non-insulin dependent diabetes mellitus. Hence in this research, moderate diabetes was produced by Alloxan monohydrate in a dose of 135 mg/kg body weight.

In light of results, the study indicates that the ACE inhibitor Enalapril in a dose of 3.6 mg/kg body weight did not exhibit significant reduction in blood glucose levels in normoglycemic rats but it showed significant anti-hyperglycemic activity in diabetic rats where it enhanced the glucose lowering capacity of all three Oral anti-diabetic drugs (Metformin, Glimepiride and Pioglitazone). The Metformin delays the absorption of glucose from the gastrointestinal tract, increases the insulin sensitivity of cells, suppresses hepatic gluconeogenesis and enhances glucose transport in fat and muscle. It does not usually lower blood glucose concentrations in non-diabetic subjects. The Glimepiride induce insulin secretion in the pancreatic beta cells and inhibit glucagonolysis and gluconeogenesis in the liver. Through improving insulin binding to surface receptors, they also enhance the insulin sensitivity of target cells. Furthermore, there is in vitro evidence for an increased number of insulin receptors in tissues of action. The Pioglitazone has demonstrated improvements in insulin sensitivity and the ability to lower fasting plasma glucose levels. The present findings suggest that Enalapril may have some insulin sensitivity potentiating properties which are seen in type 2 diabetes mellitus but not in normoglycemic rats. Eleven studies document the potential for ACE inhibitor-associated hypoglycemia, and a majority of these are with the use of captopril.

Although the present findings confirm the anti-hyperglycemic potential but the precise mechanism of its action requires further studies for appropriate elucidation. One study demonstrates that ACE inhibitors have no impact on hepatic or peripheral insulin sensitivity. Because of the contradicting information available, the actual impact of ACE inhibitors on blood glucose is not clear. The ACE inhibitors are the drug of choice of diabetic nephropathy and diabetic hypertension and are frequently used with oral anti-diabetic drugs. They attack a fundamental abnormality in the hypertensive kidney disease and provide nearly uniform renal protective effect along with blood pressure control. It is important to evaluate clear cut picture of ACE inhibitors on blood glucose level in large sample size with ultra modern scientific techniques to reduce the hypoglycemic episode in diabetic individuals.
CONCLUSION

In the present study it is evident that the use of Enalapril has anti-hyperglycemic activity as it lowers serum glucose levels and significantly increases glucose tolerance in diabetic rats. Moreover it may also induce anti-hyperglycemic effect of oral anti-diabetic drugs in experimental animal. It is therefore advisable to prescribe and use of ACE inhibitors with anti-diabetic drugs carefully with necessary dose adjustment to avoid adverse hypoglycemic episodes in diabetic individuals. Further studies are necessary to substantiate the above observation and to work out the exact mechanism of action involved in the anti-hyperglycemic activity of ACE inhibitors.

Table 1: Effect of Enalapril on blood glucose level in normal rats.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Groups</th>
<th>Serum glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min (Fasting)</td>
</tr>
<tr>
<td>1</td>
<td>I(NC)</td>
<td>78.33±3.19</td>
</tr>
<tr>
<td>2</td>
<td>II(NE)</td>
<td>75.16±2.77</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E (% reduction); (n=6), Significance levels as compared to control (*P≤0.05  **P≤0.01  ***P≤0.001)

Table 2: Effect of Oral anti-diabetic drugs on blood glucose level in Alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Groups</th>
<th>Serum glucose level (mg/dl)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0 min (Fasting)</td>
</tr>
<tr>
<td>1</td>
<td>IV(D+M)</td>
<td>224.00±3.69</td>
</tr>
<tr>
<td>2</td>
<td>VII(D+M+E)</td>
<td>123.50±1.52</td>
</tr>
<tr>
<td>3</td>
<td>V(D+G)</td>
<td>226.66±5.03</td>
</tr>
<tr>
<td>4</td>
<td>VII(D+G+E)</td>
<td>131.50±1.68</td>
</tr>
<tr>
<td>5</td>
<td>VI(D+P)</td>
<td>226.83±3.58</td>
</tr>
<tr>
<td>6</td>
<td>IX(D+P+E)</td>
<td>128.16±5.12</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E (% reduction); (n=6), Significance levels as compared to control (*P≤0.05  **P≤0.01  ***P≤0.001)

Table 3: Interaction of Enalapril with the Oral hypoglycemic drugs in Alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Groups</th>
<th>Serum glucose level (mg/dl)</th>
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