

EFFECTS OF ENALAPRIL ON BLOOD GLUCOSE LEVEL AND INTERACTION WITH THE ORAL ANTI-DIABETIC DRUGS IN ALLOXAN-INDUCED DIABETIC RATSNEERAJ K. AGRAWAL^{1*}, UMA GUPTA², S.P.SINGH³

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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 The 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 diabetes⁴⁻⁵. Diabetic nephropathy typically present as microalbuminuria before progressing to macroalbuminuria, a decrease in creatinine clearance and finally renal failure⁶. 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 type⁷⁻⁸.

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 nephropathy⁹. 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, GSVM 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 pellet diet and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee, GSVM Medical College, and 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)¹⁰. 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- Gliclazide (22 mg/ Kg body weight/day /rat) (c) Peroxisome Proliferator-Activated Receptor-gamma (PPAR- γ) 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 Gliclazide 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+E) with drugs Enalapril and Gliclazide 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)¹¹ 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 puncture¹². 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. The plasma blood glucose levels were determined by using GOD-POD method¹³. Span diagnostic reagent kit (code no. B 0112) was used for estimation of blood glucose level.

Statistical analysis

Data were expressed as mean \pm 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).

RESULTS

Effect on Normal Rats

Effect of Enalapril on blood glucose levels in normal rats outlined in table 01 which is showing percentage changes in serum glucose levels and significance which is expressed with respect to control group. It is observed that the drug Enalapril in a dose of 3.6 mg/ kg body weight slightly reduced the blood glucose level at all time points but it is not significant (P>.005). It means, it has some hypoglycemic activity in normal rats which is insignificant.

Effect on Alloxan Induced Diabetic Rats

The effect of 13-day administration of Oral anti-diabetic drugs on blood glucose levels in Alloxan induced diabetic rats is shown in Table 02. The results indicate that all three drugs Metformin, Gliclazide and Pioglitazone have significant hypoglycemic activity at all time points in Alloxan induced diabetic rats.

The Interaction of Enalapril with the Oral hypoglycemic drugs in

Alloxan induced diabetic rats outlined in table 03 which is also showing percentage changes in serum glucose levels and significance which is expressed with respect to positive control groups. It revealed that blood glucose level is decreased with the drug Enalapril at all time points in all groups which is highly significant (P \leq 0.001). In comparison of group IV (D+M) and group VII (D+M+E), Enalapril exhibited highest hypoglycemic activity at '120' min time point with reduction of 45.44% whereas in group V (D+G) & group VIII (D+G+E) and group VI (D+P) & group IX (D+P+E), there were highest impact on '0' min (fasting) with reduction of 41.98% and 43.49% respectively.

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 animals¹⁴. 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 cells¹⁵. 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 content¹⁶. The Moderate diabetic animals are recommended for use in testing drugs for use in Non-insulin dependent diabetes mellitus¹⁷. 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, Gliclazide and Pioglitazone). The Metformin delays the absorption of glucose from the gastrointestinal tract, increases the insulin sensitivity of cells, suppresses hepatic gluconeogenesis¹⁸⁻¹⁹ and enhances glucose transport in fat and muscle²⁰. It does not usually lower blood glucose concentrations in non-diabetic subjects. The Gliclazide induce insulin secretion in the pancreatic beta cells and inhibit gluconeogenesis 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 action²¹. The pioglitazone has demonstrated improvements in insulin sensitivity and the ability to lower fasting plasma glucose levels²². 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 captopril²³⁻²⁴. Though the mechanism for this drug-induced hypoglycaemia is not well defined, it is proposed that the increase in bradykinins associated with ACE inhibitor use may cause an increase in insulin sensitivity²⁵⁻²⁶.

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 sensitivity²⁷. 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 control²⁸⁻²⁹. Hence 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 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.

S.N.	Groups	Serum glucose level (mg/dl)			
		0 min(Fasting)	30 min	60 min	120 min
1	I(NC)	78.33±3.19	100.66±2.71	91.50±1.66	85.66±2.98
2	II(NE)	75.16±2.77(4.04%)	95.33±1.45(5.29%)	89.00±1.31(2.73%)	80.83±1.83(5.63%)

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

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

S.N.	Groups	Serum glucose level (mg/dl)			
		0 min(Fasting)	30 min	60 min	120 min
1	III(DC)	254.16±6.12	286.16±5.33	345.83±8.10	350.83±6.71
2	IV(D+M)	224.00±3.69 ^b (11.86%)	254.83±8.85 ^a (10.94%)	288.83±6.30 ^c (16.48%)	228.50±5.28 ^c (34.86%)
3	V(D+G)	226.66±5.03 ^b (10.81%)	256.83±9.11 ^a (10.24%)	290.16±0.83 ^c (16.09%)	229.50±6.09 ^c (34.58%)
4	VI(D+P)	226.83±5.38 ^b (10.75%)	256.56±8.63 ^a (10.34%)	293.00±2.39 ^c (15.27%)	230.33±5.25 ^c (34.34%)

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

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

S.N.	Groups	Serum glucose level (mg/dl)			
		0 min(Fasting)	30 min	60 min	120 min
1	IV(D+M)	224.00±3.69	254.83±8.85	288.83±6.30	228.50±5.28
2	VII(D+M+E)	123.50±1.52 ^c (44.86%)	166.66±3.43 ^c (34.59%)	197.16±3.74 ^c (31.73%)	124.66±2.87 ^c (45.44%)
3	V(D+G)	226.66±5.03	256.83±9.11	290.16±0.83	229.50±6.09 ^c
4	VIII(D+G+E)	131.50±1.68 ^c (41.98%)	169.83±4.75 ^c (33.87%)	212.33±3.06 ^c (26.82%)	146.00±2.94 ^c (36.38%)
5	VI(D+P)	226.83±5.38	256.56±8.63	293.00±2.39	230.33±5.25
6	IX(D+P+E)	128.16±5.12 ^c (43.49%)	206.83±5.17 ^c (19.38%)	176.00±5.40 ^c (39.93%)	134.00±4.25 ^c (41.82%)

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

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