INFLUENCE OF BENAZEPRIL ON GLUCOSE LOWERING EFFECT OF GLIPIZIDE IN NORMAL AND DIABETIC RATS

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Summary

The aim of the present work was to assess the influence of benazepril on the glipizide action in healthy and streptozotocin induced diabetic albino rats using single and repeated treatments of selected doses (3mg, 6mg and 12 mg/kg) of benazepril on the glucose lowering effect of glipizide. The whole study was divided into 3 phases. In the first phase the effect of benazepril (3mg/kg, 6mg/kg and 12mg/kg, p.o) and glipizide (10mg/kg) individually were on the blood glucose levels were established in normal healthy rats. In the second phase the effect of single and repeated treatments (7 days) of benazepril on the hypoglycemic effect of glipizide in healthy rats were studied. In the third phase the possibility of drug-drug interaction between benazepril and glipizide in diabetic conditions were explored by following the above procedure using STZ induced diabetic rats.
Blood samples were collected from tail vein initially at '0' hour and following drug administration at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 18.0, 24.0 and 30.0 hours for estimation of blood glucose. Same above experimental protocol was repeated in STZ induced diabetic rats and blood glucose levels were analyzed as mentioned earlier. Benazepril significantly altered the peak effect and enhanced the hypoglycemic activity in both normal and diabetic animals following treatment for 7 days without any change in onset of action of glipizide. However there were no significant effects observed following a single day treatment of benazepril on glipizide. The study suggests that, the dose and frequency of glipizide must be readjusted when it is to be used concomitantly with antihypertensive drug benazepril in combined hypertension and diabetic conditions to avoid severe hypoglycemia due to benazepril.

Key words: Glipizide; benazepril; hypoglycemic; antidiabetic; drug- drug interaction.

Running title: INFLUENCE OF BENAZEPRIL ON GLIPIZIDE ACTION IN RATS

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Introduction

Hypertension and diabetes mellitus are common chronic conditions which frequently coexist and can significantly affect individual health care needs. The prevalence of hypertension increases with age and is common in both cases of insulin-dependent diabetes mellitus (IDDM) and non insulin-dependent diabetes mellitus (NIDDM) conditions. Hypertension appears to be critically important in diabetes mellitus, not only because of its increased prevalence, but also it accelerates both the microvascular and macrovascular complications of diabetes.

Combination of hypertension and diabetes mellitus produces greater myocardial dysfunction and is associated with significant mortality rates. Hence controlling blood pressure in diabetics is positively more beneficial as far as progressions of diabetic complications are concerned[1]. In such conditions, there is a need for the use of antihypertensive drugs like benazepril (ACEI) with antidiabetic drugs like glipizide arises. Such combinations may lead to drug-drug interactions, which can be defined as the modifications of the effects of first drug i.e. the object drug by the prior or concomitant administration of second drug i.e. the precipitant or vice versa[2]. Recent studies have suggested that ACE inhibitors may play an important role in the prevention of type II diabetes. This is especially important because many common cardiovascular conditions such as coronary disease, congestive heart failure and hypertension are associated with insulin resistance and increased risk for the development of diabetes[3,4, 5].

The risk of hypoglycemia increases many folds in patients taking insulin or hypoglycemic agent with ACEI (angiotensin converting enzyme inhibitors). Hence it is important to discuss about the occurrence and management of potential drug-drug interactions to bring awareness amongst the health care professionals[6]. Present study was carried with the objective of establishing the effect of benazepril on glipizide action.

Materials and Methods

Animals

Wistar albino rats of either sex (175-250g) were procured from Sri Venkateswara Enterprises, Bangalore, and were maintained under standard husbandry conditions (temperature of 250± 1^0C; RH 45 to 55% and 12: 12 light/dark cycle). Animals were fasted for 18 hours before commencing the experiment. During this period, they were given water *ad libitum*. The fasting was continued till the completion of the experiment. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of V.L. College of pharmacy, Raichur, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and Chemicals

Glipizide was obtained as a gift sample from Sun pharma ltd., Mumbai and Benazepril as gift sample from Aurobindo Pharma ltd., Hyderabad, AP, India.

Streptozotocin was purchased from Sigma-Aldrich, Bangalore. Kit for biochemical estimation of glucose was procured from Span Diagnostic Ltd and estimation was carried by GOD/POD method using semi autoanalyser (ERBA mannheim, CHEM- 5 plus v_2).
The percentage reduction in blood glucose levels at time "t" was calculated by using the following equation.

Percentage of Blood glucose reduction at time't'

Where,

$$\frac{A-B}{A} \times 100$$

A= Initial blood glucose level before drug administration.

B= Blood glucose levels at time "t" after the drug administration.

Experimental design

The whole study was divided into 3 phases. In the first phase the effect of benazepril (3mg/kg, 6mg/kg and 12mg/kg, p.o) and glipizide (10mg/kg, p.o) were established individually on the blood glucose levels in normal healthy rats. In the second phase effect of single day treatment of benazepril (3mg, 6mg and 12mg/kg, p.o) and repeated treatment of benazepril (3mg, 6mg and 12mg/kg, p.o) for 7 days on the hypoglycemic effect of glipizide (10mg/kg, p.o) in healthy rats were studied.

In the third phase the possibility of drug-drug interaction between benazepril and glipizide in diabetic conditions were explored by following the above procedure using STZ induced diabetic rats.

'0' hr blood samples were collected for fasting blood glucose levels estimation. Benazepril (3mg/kg, p.o) and Glipizide (10mg/kg, p.o) was administered orally to all the rats and the blood samples were collected at prefixed time intervals 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 18.0, 24.0 and 30.0 hrs and were analyzed for glucose levels.

Induction of diabetes

Rats of either sex weighing (175-200 gms) were selected and fasted for 18 hours prior to experiment and water supplied ad-libitum. The rats were administered with 65mg/kg of Streptozotocin intraperitoneally which was prepared freshly at the time of administration in citrate buffer (pH 4.4, 0.1M) and was used within 10 minutes of its preparation. After one week, the blood samples were collected and analysed for blood glucose levels. Rats with blood glucose levels more than 200md/dl were included in the experiment. In our experiment diabetes was characterized by weight loss and hyperglycemia.86 and these animals were used for antidiabetic study.

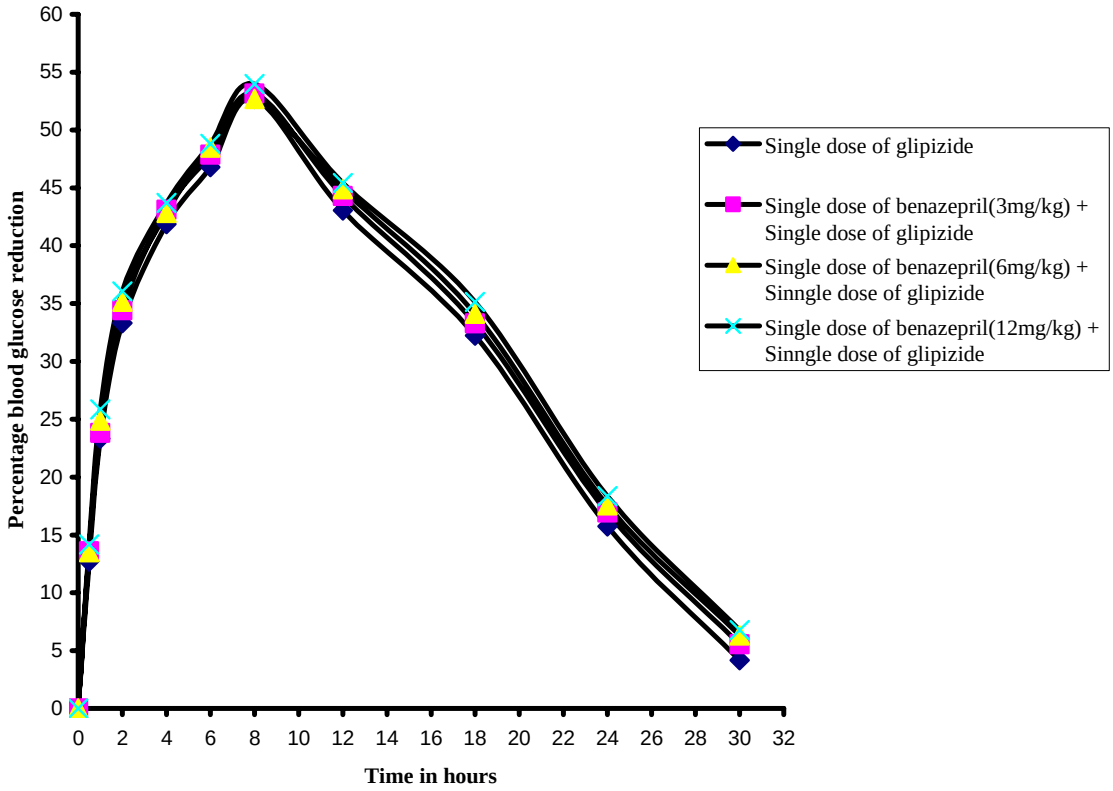
Statistical analysis

Results are expressed as mean ± SEM. Statistical differences between means were analyzed using one-way ANOVA followed by Bonferroni multiple comparison test and P< 0.001 was considered as significant. The statistical analysis was performed using demo version of Instat® software (Graph pad Inc., Santabara, CA).

Results

Benazepril when given as a single dose (3mg/kg, 6mg/kg and 12mg/kg, p.o) has not shown significant alteration in hypoglycemic effect of glipizide in normal animals (Fig 1). However repeated treatment with benazepril for a period of 8 days at doses of 6mg/kg and 12mg/kg have significantly (p<0.001) altered the hypoglycemic activity of glipizide from 0.5 to 8 hr in normal rats (Table 1).

Fig1: Effect of single dose treatment of bennazepril (3, 6 and 12mg/kg) on hypoglycemic activity of glipizide in healthy rats



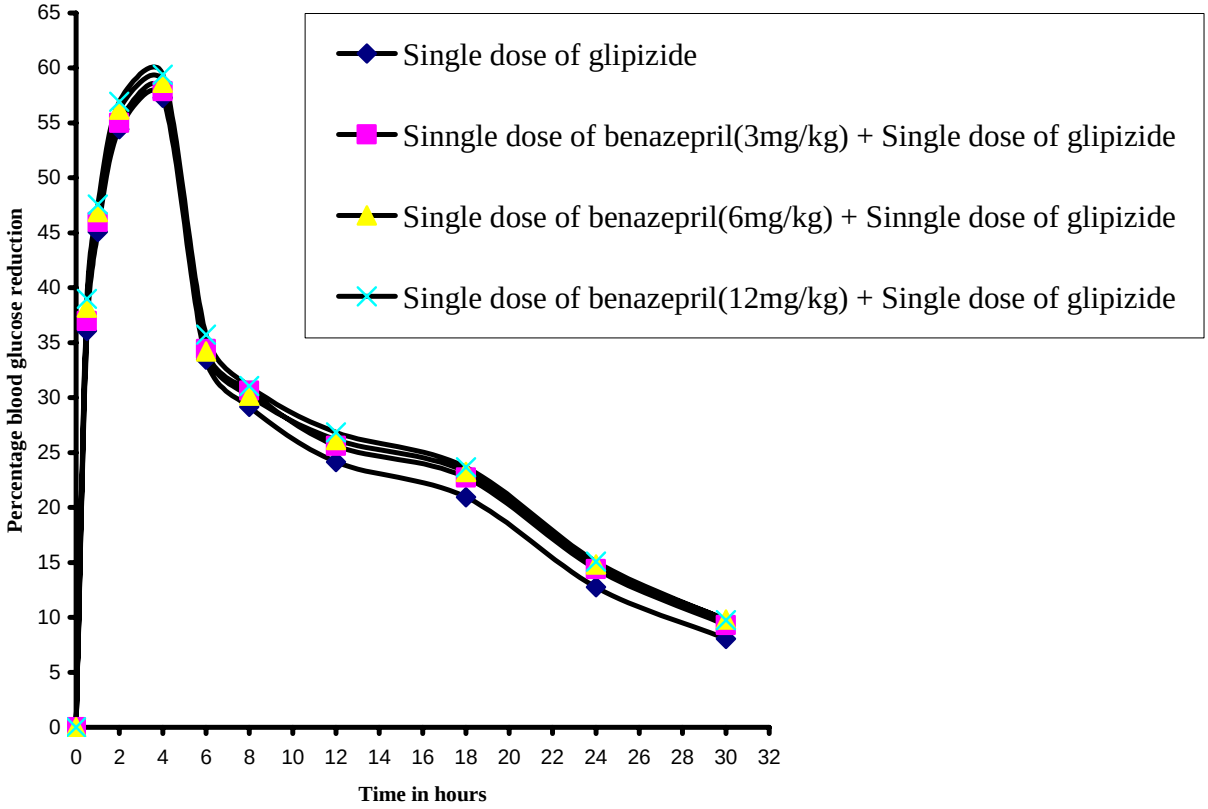
Like wise effect of single dose treatment of benazepril (3mg/kg, 6mg/kg and 12mg/kg, p.o) on antidiabetic activity of glipizide was studied in streptozotocin induced diabetic rats. No significant alteration in antidiabetic activity of glipizide was observed throughout the experimental period (Fig 2). However repeated treatment with benazepril for a period of 8 days at doses of 6mg/kg and 12mg/kg have significantly ($p<0.001$) altered the antidiabetic activity of glipizide from 0.5 to 8 hr in diabetic rats (Table 2).

Table1: Effect of repeated dose treatment of benazepril (3 mg/kg, 6 mg/kg and 12 mg/kg, p.o) for 7 days on hypoglycemic activity of glipizide (10 mg/kg, p.o) in healthy albino rats

Time in hours	Percentage blood glucose reduction				
	Benazepril (3 mg/kg, p.o)	Glipizide (10mg/kg, p.o)	Repeated dose treatment of benazepril (3 mg/kg) + Single dose of glipizide (10mg/kg)	Repeated dose treatment of benazepril (6 mg/kg) + Single dose of glipizide (10 mg/kg)	Repeated dose treatment of benazepril (12 mg/kg) + Single dose of glipizide (10 mg/kg)
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
Fasting	0	0	0	0	0
0.5	4.60±0.31	12.70±0.96	13.84±0.44	17.87±0.37	20.63±0.55***
1.0	10.71±0.47	23.33±0.78	25.12±0.84	30.59±0.72***	35.55±0.41***
2.0	14.26±0.34	33.31±0.56	36.51±0.73	43.05±0.90***	46.60±0.22***
4.0	12.12±0.29	41.86±0.87	43.90±1.08	47.80±0.80**	51.51±0.43***
6.0	10.18±0.20	46.78±0.77	49.00±0.69	50.54±0.60	55.02±0.77***
8.0	7.80±0.22	52.68±0.44	53.84±0.33	53.45±0.59	56.52±0.55
12.0	5.88±0.25	43.05±0.95	45.52±0.81	45.77±0.91	47.72±1.07
18.0	3.12±0.10	32.22±1.12	34.25±0.95	35.24±1.34	36.05±1.29
24.0	2.03±0.27	15.73±1.28	17.87±1.34	18.44±1.46	18.84±1.59
30.0	0.25±0.19	4.17±0.88	6.08±1.06	7.00±1.07	7.08±0.93

Results are expressed as mean ± SEM from 6 animals. Significant at $p< 0.05^{*}$, 0.01^{**} and 0.001^{***}

Fig 2: Efect of single dose of benazepril (3, 6 and 12mg/kg) on antidiabetic activity of glipizide in diabetic rats



Discussion

The present study was undertaken to verify the possible interaction if any between single and repeated dose treatment of benazepril on hypoglycaemic and antidiabetic activity of glipizide in both healthy and diabetic rats. Parameters considered for the study were the onset and duration of hypoglycaemic action of glipizide. Single and repeated dose pretreatment of benazepril have shown significant hypoglycaemia, exhibited by reduced activity (movement), confined to a single place of the case with signs of depression which was further confirmed by significantly enhanced duration of hypoglycaemic activity of glipizide and slight alteration in peak effect. These results suggest that, during concomitant administration of glipizide and diltiazem, the dose and frequency of glipizide need to be readjusted accordingly. In addition, monitoring of regular blood glucose levels during that period is essential to avoid the unwanted complications like severe hypoglycaemia and convulsions.

Table2: Effect of repeated dose treatment of benazepril (3mg/kg, 6mg/kg and 12mg/kg, p.o) on antidiabetic activity of glipizide (10mg/kg, p.o) in diabetic albino rats

Time in hours	Percentage blood glucose reduction				
	Benazepril (3mg/kg, p.o)	Glipizide (10mg/kg, p.o)	Repeated dose treatment of benazepril (3mg/kg) + Single dose of glipizide (10mg/kg)	Repeated dose treatment of benazepril (6mg/kg) + Single dose of glipizide (10mg/kg	Repeated dose treatment of benazepril (12mg/kg) + Single dose of glipizide (10mg/kg
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
Fasting	0	0	0	0	0
0.5	3.37±0.27	36.06±0.72	38.02±0.84	43.16±0.68***	45.59±0.28***
1.0	6.54±0.32	45.07±0.80	47.04±0.96	50.30±0.78***	51.31±0.16***
2.0	8.87±0.35	54.44±0.74	55.71±0.82	60.59±0.40***	61.77±0.30***
4.0	12.48±0.21	57.28±0.64	58.43±0.60	63.23±0.42***	70.04±0.23***
6.0	8.55±0.08	33.47±0.48	35.10±0.39	37.64±0.41**	48.67±0.30***
8.0	6.93±0.11	29.15±0.57	31.59±0.75	31.07±0.63	35.39±0.39***
12.0	5.85±0.17	24.15±1.02	26.61±1.23	26.61±1.21	27.22±0.33***
18.0	4.40±0.12	20.94±0.99	24.09±0.76	24.09±0.70	24.30±1.16
24.0	1.99±0.26	12.57±1.26	15.41±1.17	15.93±1.19	17.06±0.84*
30.0	0.46±0.03	8.06±0.65	10.61±0.82	10.45±0.82	10.83±0.99

Results are expressed as mean ± SEM from 6 animals. Significant at $p< 0.05^{*}$, 0.01^{**} and 0.001^{***}

From the earlier reports it was noted that ACEI has increased the blood glucose levels by decreasing serum potassium levels[7] there by inhibiting insulin release [8, 9] and overall inhibition of glucose uptake by peripheral tissues [10] in normal rats and our results are similar with the reports. Several studies have shown that ACE inhibitors and ARBs decrease the incidence of new-onset type 2 diabetes by their possible protective effect on the pancreatic beta cell through inhibiting the vasoconstrictive effect of angiotensin II in the pancreas and increasing islet blood flow, which could improve insulin release by beta cells[11].

Clinically ACE inhibitors are one of the drugs of choice in patients who are diabetic with mild to severe hypertension. Concomitant administration of ACE inhibitors with oral hypoglycemic agents or insulin therapy has been reported. The risk of hypoglycemic was increased several folds in patients taking insulin or hypoglycemic agent with ACE inhibitors. ACE inhibitors could improve the blood circulation in skeletal muscles, thus favoring peripheral insulin action, but also in the pancreas, thus promoting insulin secretion [12, 13]. Hence the alteration of glipizide action by benazepril may be contributed to its ability to alter potassium levels or its protective effect on pancreatic beta cells. However, the results are yet to be confirmed by understanding pharmacokinetic parameters like AUC, Cmax and tmax of glipizide after treatment with single and repeated doses of benazepril.

Conclusion

The present study suggests that during simultaneous treatment for hypertension and diabetes with benazepril and glipizide the dose and frequency of glipizide has to be readjusted accordingly to avoid severe hypoglycemia.

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