INFLUENCE OF TRANDOLAPRIL TREATMENT WITH GLIPIZIDE ON SERUM GLUCOSE AND POTASSIUM LEVELS IN NORMAL AND DIABETIC RATS

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Summary

The aim of the study was to assess the influence of trandolapril on glipizide action in normal and diabetic rats using single and repeated dose treatments of selected doses of trandolapril. In this study blood samples were collected from tail vein for estimation of serum glucose and potassium. Trandolapril significantly altered the peak effect and enhanced the hypoglycemic activity in both normal and diabetic rats at single and repeated dose treatment. It has also increased the serum potassium levels. This suggests that, the dose and frequency of glipizide must be readjusted when it is to be used concomitantly with trandolapril.

Key words: Glipizide; trandolapril; glucose; potassium; hypoglycemic.
Introduction

Diabetes mellitus is estimated to be the fifth leading cause of death globally and the risk of cardiovascular disease is higher in diabetic subjects compared to non-diabetics. Diabetes is a growing problem, in 2003 it was estimated that approximately 194 million people had diabetes, in 2007 the total was 246 million. Of these, the majority (90%) had type 2 diabetes mellitus (T2DM). By 2025, the number is expected to rise to 380 million.

Insulin resistance plays a causal role in hypertension and atherosclerosis, and thus is present to some degree in most patients with these diseases. About 50% of hypertensive individuals are hyperinsulinemic and up to 75% of people with type 2 diabetes have hypertension. Abnormal glucose metabolism is seen in approximately two of three patients presenting with an acute coronary syndrome (with about equal numbers of patients having impaired fasting glucose and overt diabetes).

Recent studies indicate that ACE inhibitors can improve insulin sensitivity during either short-term or long-term administration. Rett et al. reported that insulin sensitivity increased in normotensive patients with NIDDM about 20 min after oral administration of captopril. Torlone et al. reported that a 2-day treatment with captopril improved insulin sensitivity in hypertensive NIDDM patients. Rett et al. have suggested the contribution of bradykinin, although this is still not widely accepted.

An increased incidence of severe hypoglycemic episodes in patients with type 2 diabetes treated with ACE inhibitors was previously described in some case-control studies. Interestingly, the highest risk of severe hypoglycemia among all patients on oral antidiabetic agents observed was in those patients concomitantly treated with ACE inhibitors and oral hypoglycemic agents. Apart from systematic studies, a number of case reports seem to confirm an increased risk of hypoglycemia in patients with diabetes treated with ACE inhibitors. For example, Arauz-Pacheco and colleagues reported two patients with stable noninsulin dependent diabetes treated with sulfonylurea in whom hypoglycemic episodes occurred almost immediately after the initiation of ACE-inhibitor therapy for hypertension. Conversely, no episodes had occurred in these patients earlier, when on glibenclamide monotherapy.
The mechanisms leading to hypoglycemic episodes in the presence of ACE inhibitors are still a matter of debate. Some authors suggest an increase of insulin sensitivity in patients with type 2 diabetes due to ACE inhibitor use, but these findings are controversial. Furthermore, a randomized, controlled, doubleblind study investigating the effects of ACE inhibitors compared to placebo on insulin sensitivity remains to be published. Other than improved sensitivity to insulin, altered pharmacokinetic or pharmacodynamic properties of the antidiabetic drug might be another reason for the observed increased lowering of blood glucose levels. Therefore, the goal of this study was to evaluate whether pharmacokinetic or pharmacodynamic interactions exist when enalapril is coadministered with glibenclamide in healthy, normotensive volunteers.4

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°C; RH 45 to 55% and 12: 12 light/dark cycle). Animals were on fasting 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 experiment was performed after prior approval of the study protocol by the institutional animal ethical 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 Trandolapril as gift sample from Medreich Sterilab Ltd., Bangalore, Karnataaka, India. Streptozotocin was purchased from Sigma-Aldrich, Bangalore. Kit for biochemical estimation of glucose was procured from Span diagnostics Ltd. Sachin (Surat), India and estimation was carried by GOD/POD method using semi autoanalyser (ERBA mannheim, CHEM- 5 plus v2). Kit for biochemical estimation of potassium was procured
from Crest biosystems, Goa, India and estimation was carried by turbidometric method using semi autoanalyser (ERBA mannheim, CHEM- 5 plus v₂).

The percentage reduction in serum glucose and potassium levels at time “t” was calculated by using the following equation.

**Percentage reduction in serum glucose at time “t”**

\[ t = \frac{A - B}{A} \times 100 \]

Where \( A \) is serum glucose concentration at time “0” and

\( B \) is serum glucose concentration at time “t”.

**Percentage increase in serum potassium at time “t”**

\[ t = \frac{B - A}{A} \times 100 \]

Where \( A \) is serum potassium concentration at time “0” and

\( B \) is serum potassium concentration at time “t”.

**Experimental design:**

The whole study was divided into 3 phases. In I phase the effect of trandolapril (low dose, LD 2.5 mg/kg, intermediate dose, ID 5 mg/kg, medium dose, MD 10 mg/kg and high dose, HD 12 mg/kg p.o) and glipizide (10 mg/kg, p.o) were established individually on the serum glucose and potassium levels in normal rats. In II phase effect of single day treatment of trandolapril (LD 2.5 mg/kg, ID 5 mg/kg, MD 10 mg/kg and HD 12 mg/kg, p.o) and repeated treatment of trandolapril (LD 2.5 mg/kg, ID 5 mg/kg, MD 10 mg/kg and HD 12 mg/kg, p.o) for 7 days on the serum glucose and potassium levels in normal rats were studied.

In III phase the possibility of drug-drug interaction between trandolapril and glipizide in diabetic conditions were explored by following the above procedure using streptozotocin (STZ) induced diabetic rats.

‘0’ h blood samples were collected for fasting serum glucose and potassium levels estimation. Trandolapril (p.o) and Glipizide (p.o) was administered orally to all the rats and the blood samples were collected at prefixed time intervals i.e. 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 18.0, 24.0 and 30.0 h and were analyzed for serum glucose and potassium levels.
Induction of diabetes:

Rats of either sex weighing (175-200g) were selected and fasted for 18 hours prior to experiment and water supplied ad-libitum. The rats were administered with 65mg/kg of Streptozotocin i.p. 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 analyzed for serum glucose levels. Rats with serum glucose levels more than 200 mg/dl were included in the experiment. In our experiment diabetes was characterized by weight loss and hyperglycemia 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 Dunnett’s-“t” test and P< 0.05 was considered as significant. The statistical analysis was performed using demo version of Instat® software (Graph pad Inc., Santabara, CA).

Results

In the first phase of the study in different groups of normal rats the effect of single dose treatment of trandolapril LD, ID, MD and HD and glipizide (10 mg/kg, p.o) on serum glucose levels were studied. Trandolapril 2.5 mg/kg, p.o. (LD), 5 mg/kg, p.o. (ID) when administered as single doses to normal rats it has significantly reduced serum glucose levels at 2, 4, 6, 8 12 and 18th h of the experimental study. Trandolapril with MD and HD i.e.10 mg/kg, p.o. and 20 mg/kg HD, p.o when administered as single doses have significantly reduced the serum glucose levels through out the experimental study except at 0.5 h. Glipizide has produced a significant hypoglycemic effect and the peak hypoglycemic effect was observed at 6th h.

Trandolapril LD, ID, MD and HD have not altered the onset of action of glipizide, but slightly increased peak effect with no change in duration of glipizide in normal rats. After repeated treatment with trandolapril LD, ID, MD and HD for 7 days it has not altered the onset of hypoglycemic action of glipizide but with a positive influence on the peak hypoglycemic effect and a significant enhancement in the duration of action.
Repeated treatment of Trandolapril LD and ID administered for 7 days followed by single dose of glipizide has not altered the hypoglycemic activity of glipizide up to 1 h but later there was a significant decrease in the serum glucose levels at 2, 6, 8, 12 $^{th}$ h. Similarly, repeated Trandolapril MD treated group had shown significant serum glucose reduction from 2 to 18 $^{th}$ h and also at 24 $^{th}$ h of the study period and HD had shown its significant hypoglycemic effect on glipizide from 2$^{nd}$ h till the end of the experimental period (fig 1 and 2).

In diabetic rats, glipizide as a single dose exhibited significant serum glucose reduction from 0.5 to 18 h of experimental study and the peak antidiabetic effect was at observed at 6 $^{th}$ h. Single dose of treatment of Trandolapril (LD) in diabetic rats has produced significant decrease in serum glucose levels from 2 to 12 $^{th}$ h, ID from 1 to 12 $^{th}$ h, MD from 2 to 12 $^{th}$ h and HD produced significant decrease in serum glucose levels from 1 to 18 $^{th}$ h of experimental study.

In case of diabetic rats, single dose treatment of Trandolapril LD followed by single dose of glipizide has shown significant increase in the antidiabetic effect of glipizide at 2, 4, 12, 24 and 30$^{th}$ h. Trandolapril ID single dose followed by single dose of glipizide had produced significant increase in antidiabetic activity of glipizide from 2$^{nd}$ hour to the end of the study period except at 8 h. Trandolapril MD and HD as single doses followed by single dose of Glipizide has shown significant antidiabetic activity of glipizide from 1st h of administration of glipizide to the end of the study period (fig 3).

In case of repeated treatment of various doses of Trandolapril, LD has shown significant increase in antidiabetic activity of glipizide through out the study period except at 1 and 30 $^{th}$ h. Repeated administration of ID for 7 days has significantly increased the antidiabetic effect of glipizide through out the study period except at 18$^{th}$ h. Similarly, repeated treatment of MD and HD for 7 days has significantly altered antidiabetic effect of single dose of glipizide through out the study period (fig 4).

In normal rats single doses of Trandolapril LD, ID, MD and HD followed by single dose of glipizide treatment significantly increased the serum potassium levels from 4$^{th}$ h till the completion of the study. In this case Trandolapril LD has increased at 4, 6, 8, 12 and 24 $^{th}$ h, ID and MD at 4, 6, 8, 12, 18 and 24 $^{th}$ h and HD at 4, 6, 8, 12 and 18 $^{th}$ h (fig 5).
Repeated dose treatment of Trandolapril LD, ID, MD and HD followed by single dose of glipizide treated groups have shown significant increase in the potassium levels from 4\textsuperscript{th} h till the end of experimental study (fig 6).

In diabetic rats single doses of Trandolapril LD, ID and MD followed by single dose of glipizide treatment significantly increased the serum potassium levels at 2, 4, 6, 12, 18 and 24 \textsuperscript{th} h, HD has increased at 1, 2, 6, 12, 18 and 24 \textsuperscript{th} h. In repeated dose treatment of Trandolapril LD, ID, MD and HD followed by single dose of glipizide treated groups there was significant increase in the potassium levels from 1\textsuperscript{st} h till the end of experimental study (fig 7 and 8). This reveals a positive interaction between glipizide and trandolapril with concomitant treatment as the potassium levels were significantly elevated both in single and repeated dose treatment of trandolapril along with single dose of glipizide.

Fig.1. Percentage reduction in serum glucose with single dose treatment of Trandolapril (2.5,5,10 and 20 mg/kg) + glipizide (10 mg/kg) in normal rats
Fig. 2. Percentage reduction in serum glucose with repeated treatment of Trandolapril (2.5, 5, 10 and 20 mg/kg) + single dose of glipizide (10 mg/kg) in normal rats.

Fig. 3. Percentage reduction in serum glucose with single dose treatment of Trandolapril (2.5, 5, 10 and 20 mg/kg) + single dose of glipizide (10 mg/kg) in diabetic rats.
Fig. 4. Percentage reduction in serum glucose with repeated treatment of Trandolapril (2.5, 5, 10 and 20 mg/kg) + single dose of glipizide (10 mg/kg) in diabetic rats.

Fig. 5. Percentage increase in serum potassium with single dose treatment of Trandolapril (2.5, 5, 10 and 20 mg/kg) + glipizide (10 mg/kg) in normal rats.
Fig. 6. Percentage increase in serum potassium with repeated treatment of Trandolapril (2.5, 5, 10 and 20 mg/kg) + glipizide (10 mg/kg) in normal rats.

Fig. 7. Percentage increase in serum potassium with single dose treatment of Trandolapril (2.5, 5, 10 & 20 mg/kg) + glipizide (10 mg/kg) in diabetic rats.
fig. 8. percentage increase in serum potassium with repeated treatment of trandolapril (2.5, 5, 10 & 20 mg/kg) + glipizide (10 mg/kg) in diabetic rats.

Discussion

Ever since the discovery of insulin and sulfonylureas as clinically useful antidiabetic agents, control of blood sugar level with drug(s) has been the primary objective in diabetic patients. Simultaneous use of other drugs is the usual event in the life of these patients. So, drug interactions between antidiabetic agents and other classes of drugs, affecting the safety and/or efficacy of these drugs, are likely to occur. The severity of the situation is clearly reflected by data collected from the literature by Seltzer’ who recorded 473 cases of drug induced hypoglycemia of which 60 were fatal. The survey included 220 cases of hypoglycemia induced by sulfonylureas only of which 25 were fatal.6

The present study was undertaken to verify the possible drug-drug interaction if any between single and repeated dose treatment of trandolapril on hypoglycemic and antidiabetic activity of glipizide in normal and diabetic rats. The effect of trandolapril on serum potassium levels after single and repeated dose treatment was also studied.
In the present study in normal animals the effect of trandolapril on the hypoglycemic and antidiabetic activity of glipizide was studied and parameters considered for the study were onset and duration of hypoglycemic action of glipizide. After repeated dose treatment of trandolapril followed by single dose treatment of glipizide, it has shown significant hypoglycemia in all the animals, exhibited by reduced activity (movement), confined to a single place with signs of depression which was further confirmed by significant increase in duration of hypoglycemic activity of glipizide and slight alteration in peak effect after trandolapril pre treatment.

The results have indicated that trandolapril has influenced the absorption phase of glipizide. Since the peak effect and duration of hypoglycemia induced by glipizide were enhanced, so it can be concluded that trandolapril appears primarily to interfere with the absorption, protein binding or metabolism, excretion, increase in micro and macro vascular circulation to pancreas and decreased insulin resistance.

Hypertension and diabetes mellitus are two 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 micro vascular and macro vascular 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.

ACE inhibitors can help people with diabetes by reducing their risk of heart attack, stroke and premature deaths and also delay the onset and progression of kidney disease. In addition, ACE inhibitors can help to reduce other complications of diabetes such as foot ulcers and eye damage (retinopathy), as retinopathy is the leading cause of blindness for people with diabetes. People who take ACE inhibitors run a slightly increased risk of low blood sugar. Therefore, people with diabetes, should closely monitor blood sugar levels for the first few weeks after starting with ACE inhibitor, or
during an increase in the dosage of ACE inhibitor. ACE inhibitor therapy appears to improve insulin sensitivity and glucose metabolism.

Improvement in insulin sensitivity was achieved by addition of an ACE inhibitor to a tissue culture system, suggesting effects independent of the microcirculation. This improvement in insulin sensitivity at a cellular level is indicated by an increase in glucose transporter–4 protein and activity of hexokinase, a key enzyme in glucose metabolism in the skeletal muscle of obese rats treated with an ACE inhibitor. Several studies have shown that ACE inhibitors and ARBs (angiotensin receptor blockers) decrease the incidence of new-onset type 2 diabetes. A possible protective effect of ARBs and ACE inhibitors on the pancreatic β-cell through inhibiting the vasoconstrictive effect of angiotensin II in the pancreas and increasing islet blood flow, which could improve insulin release by β-cells.

ACE inhibitors increases potassium levels in the body and the same in diabetes mellitus increases effect of insulin on control of blood sugar. Concurrent use of ACE inhibitors and hypoglycemic agents usually appears to be uneventful but hypoglycemia, marked in some instances and has occurred in small number of diabetic patients taking insulin or sulfonylurea when treated with ACE inhibitors. This has been attributed, but not proved, to be due to an interaction. The problem was solved in some cases by reducing the dosage of hypoglycemic agent. 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. At the same time the risk of hypoglycemia was increased 3.5-fold 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.

Conclusion

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

1. Digital comprehensive summaries of uppsala dissertations From the faculty of pharmacy 71 Safety and efficacy modelling in Anti-diabetic drug development Bengt hamrén.
2. Hussam Abuissa, MD, Philip G. Jones, MS, Steven P. Marso, MD, James H. O’Keefe, JR, MD Kansas City, Missouri. Angiotensin-Converting Enzyme Inhibitors or Angiotensin Receptor Blockers for Prevention of Type 2 Diabetes. A Meta-Analysis of Randomized Clinical Trials. Journal of the American College of Cardiology Vol. 46, No. 5, 2005