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Influence of itraconazole on the pharmacodynamics of gliclazide in normal and diabetic rats

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ABSTRACT

The present study was aimed at investigating the effect of Itraconazole on the action of gliclazide in normal and diabetic rats. Experimental diabetes in rats was induced by injecting alloxan monohydrate (i.p) at a dose of 150 mg/kg in ice cold normal saline. The normal and diabetic rats were randomly divided into different groups and treated with Itraconazole (10mg/kg, p.o) then followed by Gliclazide (2mg/kg, p.o) alone was administered and compared with normal saline treated group. The blood samples were collected at different time intervals 1,2,3,4,6,8,10,12 h in normal and diabetic rats and the serum samples were analyzed for glucose levels. The percentage reduction in blood glucose levels were calculated with respect to initial levels. Gliclazide showed a significant reduction of elevated and normal blood glucose levels. The extent of blood glucose reduction with Gliclazide was comparatively increased in Itraconazole pretreated group. The present study results suggest that, Itraconazole enhanced the hypoglycemic activity of Gliclazide in normal rats, possibly by the inhibition of CYP2C9 enzyme (unpublished data).

Key words: Itraconazole, Gliclazide, Hypoglycemia, Alloxan monohydrate, Pharmacodynamics.

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from deficiency of insulin leading to complications involving many organs. According to WHO, in near future, the maximum increase in diabetes would occur in India. Studies in various urban areas of India revealed several fold increase in the prevalence of type 2 diabetes in the last two decades [1].

Diabetes requires lifelong treatment with drugs, diet control and exercise. Insulin is the drug of choice in type 1 diabetes and sulfonylureas are the drugs of choice in type 2 diabetes. Type 2 diabetes is more common than type 1. Sulfonylureas are most widely used drugs in type 2.The study of mechanisms of drug interaction is of much value in selecting drug concentrations to provide rational therapy [2].

Drug interaction studies assume much importance, especially for drugs that have a narrow margin of safety, and where the drugs are used for a prolonged period of time. Diabetes mellitus is one such metabolic disorder that needs treatment for prolonged periods, and maintenance of normal blood glucose level is very important in this condition, since both hyper- glycemia, as well as hypoglycemia, is unwanted phenomenon[3]. Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism, and an increased risk of complications from vascular disease [4]. Diabetes may be due to a decrease in the synthesis of insulin (type-1) or a decrease in the secretion of insulin (type-2) from the β-cells of islets of Langerhans of the pancreas [5].

Oral hypoglycemic agents are used in the treatment of type II diabetes, amongst which gliclazide, a second generation sulfonylurea derivative is preferred in the therapy because of its selective inhibitory activity towards

pancreatic K+ATP channels, antioxidant property, low incidence of producing severe hypoglycemia and other haemo- biological effects[6]. Gliclazide induces the release of insulin by triggering calcium entry into the pancreatic β cells by blocking K+ channels. Earlier studies indicate interaction of Gliclazide and many other antidiabetic drugs with several other classes of drugs [7].

Diabetic patients may also be affected with many other diseases like peptic ulcer, hypertension and more over fungal infections, which require prolong treatment. There are reports that several patients suffering from diabetes are prone to fungal infections. In such antifungal agents like Itraconazole, Fluconazole, Miconazole, Ketoconazole etc[8].

Itraconazole is highly protein bound (>99%) [9] and penetrates extensively into human tissue [10], but it has limited penetration into the cerebrospinal fluid [11]. Itraconazole is extensively metabolized by the liver, predominantly by the CYP3A4 isoenzyme system [12].

It is important to study the possible effects of itraconazole on the pharmacodynamics of Gliclazide. Reports shows that the extensive enzyme inhibition of Itraconazole mediated CYP2C9 Gliclazide metabolism. Since there is every possibility for the combined use of Gliclazide and Itraconazole in chronic diabetics. The study is planned to investigate the effect of Itraconazole on the activity of Gliclazide in normal and diabetic rats, to evaluate the safety and effectiveness of the combination. Also the study is planned to find the pharmacodynamics of Gliclazide in the presence of Itraconazole in rats.

MATERIALS AND METHODS

2.1. Drugs and chemicals:

Gliclazide and Itraconazole are gift samples from M.S.N Labs (Hyderabad, India) and LEE Pharma Ltd. (Vishakhapattanam, India). Glucose test kits (Enzymatic GOD-POD method) are purchased from local market.All other chemicals are used analytical grade.

2.2. Animals:

Albino rats of either sex, 3-4 months of age, weighing between 200 to 250 g, were used in the study. They were procured from the Sainath agencies, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 ºC and 50 ± 15% relative humidity, with a 12-h light/12-h dark cycle. Animals were fed with a *commercial pellet diet and water ad libitum*. They were fasted for 18 h prior to the experiment, and during the experiment, the food and water were withdrawn. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee (Reg. No. 51/01/C/CPCSEA/2012/14). The study was conducted in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3. Selection of doses and preparation of oral test solution/suspension:

In clinical practice, itraconazole and gliclazide in a therapeutic dose will be administered orally as antifungal and antidiabetic therapy, respectively. Human oral therapeutic doses of the respective drugs were extrapolated to rat based on body surface area [13]. But the dose of gliclazide and itraconazole for rat experiments was selected as 2 mg/kg [13] and 10 mg/kg [14] b.w. based on the influence of dose effect-relationship of gliclazide on blood glucose in normal rats. Itraconazole (10mg/kg, p.o) suspensions were prepared using 2% w/v gum [8] acacia as suspending agent. Gliclazide (2mg/kg) solution was prepared by dissolving it in a few drops of 0.1 N NaOH then made up to the volume with distilled water. All the drugs were administered to the respective groups by oral gavage [13].

2.4. Pharmacodynamic interaction study in rats:

2.4.1. Study in normal rats:

The normal rats (Group I/II/III) are used for pharmacodynamic studies and animal distribution as follows. n=6.

Group – I (Normal control) --Rats are orally administered with 0.9%w/v saline.

Group – II (Normal standard) -- Rats are orally administered with Gliclazide at a dose of 2.0 mg/kg.

Group – III (Normal test) -- Rats are fed orally with Itraconazole at a dose of 10 mg/kg followed by oral administration of Gliclazide at a dose of 2.0 mg/kg. The blood samples were withdrawn by retro-orbital plexus and by tail vein of each rat at 1, 2, 3, 4, 6, 8, 10, 12h. These blood samples were analyzed for the serum glucose levels by GOD-POD method [13,15].

2.4.2. Study in diabetic rats:

Induction of diabetes:

Diabetes was induced in rats by the administration of alloxan monohydrate in ice cold normal saline at a dose of 150 mg/kg body weight i.p. After 72hr, blood sample was collected from rats by via tail vein of all surviving animals and

the serum was analyzed for glucose levels. Rats with blood glucose levels of 300 mg/dl and above were considered as diabetic and selected for the study [16].

The diabetic groups (Group IV/V/VI) are used for the Pharmacodynamic study. And the animal distribution as follows. n=6.

Group–IV (Diabetic control)- Alloxan monohydrate 150mg/kg is given by i.p route followed by oral administration of 0.9%w/v saline.
Group–V (Diabetic standard)- Alloxan monohydrate 150mg/kg is given by i.p route followed by oral administration of Gliclazide at a dose of 2.0 mg/kg.
Group–VI (Diabetic test)-Alloxan monohydrate 150mg/kg is given by i.p route & oral administration of Itraconazole at a dose of 10mg/kg followed by oral administration of Gliclazide at a dose 2.0 mg/kg.The blood samples were withdrawn by retro-orbital plexus and by tail vein of each rat at 1, 2, 3, 4, 6, 8, 10, 12h. These blood samples were analyzed for the serum glucose levels by GOD-POD method [13].

2.5. Data and statistical analysis:

Data were expressed as mean ± SD. The significance was determined by applying Statistical analysis by One-way ANOVA followed by Tuckey Kramer Multiple comparision test.

RESULTS

The percent blood glucose reduction of gliclazide in presence and absence of itraconazole are shown in Table1, 2 and Figure 1, 2 respectively. Itraconazole enhanced the hypoglycemic activity of gliclazide in Normal Rats then compared with the diabetic group. And all the values when compared to other groups with significant results are determined.

Table 1. Mean Percentage blood glucose reduction in normal rats

TIME (hrs)	GLICLAZIDE	GLICLAZIDE ± ITRACONAZOLE
1	23.0053±0.0031	41.0051±0.0029***
2	26.0017±0.0048	52.0035±0.0043***
3	22.0064±0.0046	39.0043±0.0032***
4	19.0037±0.0020	35.0100±0.0026***
6	22.0084±0.0035	41.0066±0.0026***
8	24.0049±0.0050	46.0075±0.0039***
10	19.9960±0.0021	37.0078±0.0045***
12	11.0071±0.0065	27.0084±0.0034***

****P values < 0.0001 when compared to Gliclazide treated group.*

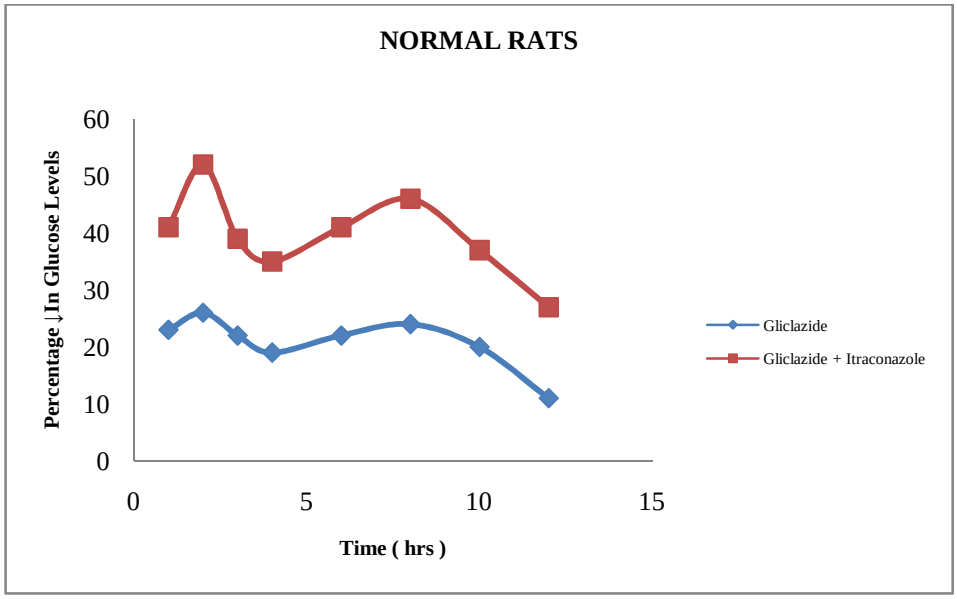

Figure 1.The percentage reduction(mg/dl) in blood glucose levels of normal rats with gliclazide before and after treatment with Itraconazole.

Table 2. Mean Percentage blood glucose reduction in Diabetic rats

TIME (hrs)	GLICLAZIDE	GLICLAZIDE ± ITRACONAZOLE
1	28.0044±0.0062	33.0043±0.0064
2	39.0014±0.0008	40.0062±0.0122
3	26.0029±0.0008	30.0024±0.0016
4	24.0013±0.0011	27.0004±0.0009
6	29.0027±0.0007	39.0017±0.0012**
8	32.0016±0.0009	35.0010±0.0007
10	27.0127±0.0293	29.0020±0.0014
12	18.0022±0.0007	21.9990±0.0034

***P values < 0.001 when compared to Gliclazide treated group.*

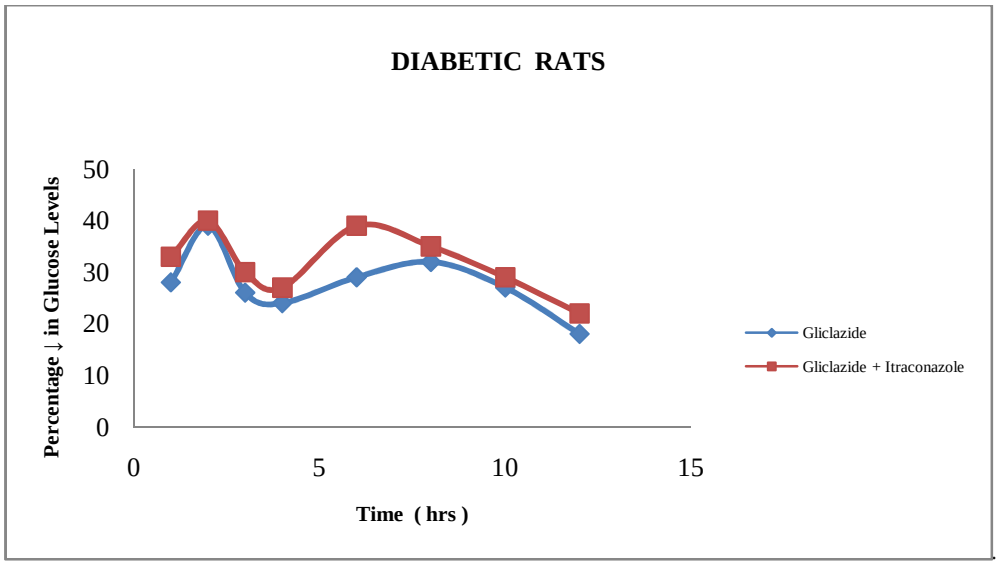

Figure 2. The percentage reduction(mg/dl) in blood glucose levels of Diabetic rats with gliclazide before and after treatment with Itraconazole

DISCUSSION

The Decreased blood glucose levels of Gliclazide, in the presence of Itraconazole is greater than when compared to the alone group of Gliclazide in Normal rats, Then the Diabetic group. It indicates Hypoglycemia condition.

Rats are known to be more sensitive to gliclazide response, and the results obtained were consistent with earlier reports from in vitro and in vivo studies. Further, the presence of interaction was supported by an increase in serum insulin levels with itraconazole treatment. It is clear that since itraconazole did not alter blood glucose levels on its own.

And also the changes observed in Ke, $t_{1/2}$, Vd , Clr indicates the metabolic level changes(Unpublished data) . Itraconazole is inhibited by CYP2C9 and CYP3A4 mediated Gliclazide metabolism ,and increased bioavailability (28.02%) leads to the decresed levels of Blood glucose levels in Gliclazide + Itraconazole combinly treated group (Unpublished data).

CONCLUSION

In conclution, Itraconazole enhanced the hypoglycemic activity of gliclazide in normal rats then compared with the diabetic treated groups. Therefore it is necessary to adopt therapeutic drug monitoring so as to readjust dose and frequency of administration of these drugs, when they are used concurrently to avoid the patients from severe hypoglycemia. The interaction appears to be pharmacodynamic interaction. which may need dosage adjustment. Hence, care should be taken when the combination is prescribed for clinical benefit in diabetic patients. However, the present study warrants further studies to find out the relevance of this interaction in human beings.

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