EFFECT OF ITRACONAZOLE ON THERAPEUTIC EFFICACY OF PIOGLITAZONE IN HEALTHY ALBINO RATS

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Summary

In the present study pretreatment of itraconazole on therapeutic efficacy of pioglitazone was evaluated in healthy albino rats. In Fasting condition, blood samples were collected by retro-orbital plexus at different time intervals for 24 hrs and blood glucose was assessed by GOD/POD method. The results were shown that itraconazole pretreatment significantly enhance the onset of hypoglycemia, peak of hypoglycemia and duration of hypoglycemic activity of pioglitazone in experimental animals. This study indicates that therapeutic drug monitoring is required to adjust therapeutic dose of itraconazole and pioglitazone when used concomitantly.

Key Words: Itraconazole, pioglitazone and hypoglycemic effect.

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Introduction

Although use of combination of drugs is a common practice for enhancing the efficiency or increasing spectrum activity of drugs, but selection of the optimal combination and the optimal doses remains a matter of trial and error.[1] A drug interaction develops when the effect of a drug is increased or decreased or when a new biological effect is developed by the prior, concurrent, or subsequent administration of the other drug. The frequency of adverse reactions is increased in the patients receiving multiple medications.[2] In patients with non-insulin-dependent diabetes, Litzelman et al reported an incidence of the following: onychomycosis (67%), tinea pedis (12.3%) interdigital infection (18.8%), ulceration (10.5%), and amputation (2.9%) etc. [3, 4] In such antidiabetic agents like sulfonylurea or thiazolidinedione or biguanides are administered along with antifungal agents like ketoconazole, itraconazole, fluconazole or clotrimazole etc.

There are reports that itraconazole is more potent than fluconazole but less potent than ketoconazole in its ability to inhibit cyclosporin metabolism.[5] Itraconazole increases the plasma concentration and effects of felodipine when it was used 200mg /day for four days.[6] Itraconazole increases plasma concentration, area under the curve and elimination half-life of oral quinidine.[7] atorvastatin[8] and tirazolam.[9] There are reports that ketoconazole interact with thiazolidinedione hypoglycemic agents and potentiate their antidiabetic activity. [10] Concomitant use of ketaconazole (an antifungal agent) and tolbutamide (an oral hypoglycemic agent) has increased potency and duration of action of tolbutamide. [11]

Pioglitazone - a thiazolidinedione class of drug acts by targeting insulin resistance by interacting with the gamma subtype of the peroxisome proliferator-activated receptor (PPAR). PPAR, a member of the nuclear receptor subfamily, stimulates gene expression of proteins involved in glucose metabolism.[12] This results in an increase in insulin sensitivity in skeletal muscle, liver, and adipose tissues.[13] The iso-enzyme CYP2C9, CYP2C8 is responsible for metabolism of thiazolidinedione (pioglitazone and rosiglitazone) [14] whereas itraconazole inhibits

mainly the CYP450 system.[15, 16] Hence the pharmacokinetics of drugs, that are metabolised by this enzyme system are normally affected by the concomitant usage of itraconazole. Itraconazole is one such relatively new antifungal agent and its interaction with pioglitazone is not reported. Hence, the present study is planned to understand possible interaction between pioglitazone and itraconazole in healthy rats.

Material and Methods

Animals

Wistar albino rats (150–200g) obtained from Sri Venkateshwara Enterprises, Bangalore. India. They were acclimatized to animal house for ten days prior to experimentation. Throughout the experiment, six animals housed per cage, and maintained at 25°C to 28°C with 50–60% relative humidity were fed standard diet with water *ad libitum.* The study was carried out as per the approval of IAEC (reg. no: 157/99/CPCSEA)**.**

Preparation of Drug solution

Pioglitazone (10 mg/kg, p.o.), Itraconazole (9 and 18mg/kg, p.o) suspensions were prepared using 2% w/v gum acacia as suspending agent.

Experimental Procedure

The animals in Group I received suspension of itraconazole (18mg/kg, p.o). Group II and group III received pioglitazone 10mg/kg per oral respectively. Blood samples were collected at 0.0, 1.0, 2.0, 4.0, 8.0, 12.0, 18.0 and 24.0 hrs after treatment by retro orbital plexus and blood glucose levels estimated by GOD/POD method were expressed as mg/dl of blood.[17]

In the next part of this experiment, animals in the Group II received suspension of itraconazole 9 mg/kg per day orally for one week. Group III received itraconazole 18 mg/kg per day orally for one week. On the 7th day, 6 hrs after administration of itraconazole the rats were fasted for 18 hrs.

On the 8th day, itraconazole 9 and 18mg/kg was administered orally to groups II and III respectively. After 60minutes, pioglitazone 10mg/kg was administered to Group II and Group III. Thereafter blood samples were collected at time intervals for 24 hrs and blood glucose levels estimated by GOD/POD method were expressed as mg/dl of blood.

Then the hypoglycemic activity of pioglitazone at time't' was calculated and the percentage of blood glucose reduction at time intervals was calculated before and after itraconazole treatment.

$$\% \text{ Blood glucose reduction at time't'} = \frac{A - B}{A} \times 100$$

Where, A = Initial blood glucose level before drug administration
B = Blood glucose level at time't' after the drug administration

Statistical data:

The data were analyzed by Student't'test. P values lower than 0.05 were considered as statistically significant.

Results

The data revealed that treatment with itraconazole alone did not alter the blood glucose levels in healthy rats (Table 1 and Fig 1). Whereas, itraconazole (9mg/kg, p.o) pretreatment altered the onset of hypoglycemic effect of pioglitazone from 25.34 ± 1.52 % at 2nd hr to 20.08 ± 2.17 % at 1st hr and significantly enhanced peak hypoglycemic effect from 33.76 ± 1.81 % to 42.80 ± 1.35 % at 4th hr and duration of hypoglycemic effect was raised for more than 24hrs. Whereas pretreatment with itraconazole (18 mg/kg, p.o) altered the onset of hypoglycemic effect of pioglitazone from 28.55 ± 2.16 % to 30.05 ± 2.38 % at 2nd hour and significantly enhanced peak hypoglycemic effect from 35.02 ± 1.23 % to 42.71 ± 2.65 % at 4th hr. Duration of hypoglycemic effect was raised for more than 24hrs (Table 2, Fig 2 and 3).

Table 1: Blood glucose level after the administration of itraconazole (18mg/kg) in healthy rats

Time in hr	Blood glucose levels (mg/dl) Mean ± SEM	Percentage blood glucose reduction Mean ± SEM
Fasting	96.81 ± 2.62	-
1.0	97.55 ± 2.43	-0.79 ± 1.18
2.0	98.34 ± 2.30	-1.63 ± 2.20
4.0	99.22 ± 2.54	-2.51 ± 1.78
8.0	100.56 ± 2.37	-3.92 ± 1.43
12.0	98.80 ± 1.90	-2.18 ± 2.31
18.0	97.60 ± 2.37	-8.50 ± 2.08
24.0	96.56 ± 2.60	-0.25 ± 1.33

Table 2: Percentage reduction in blood glucose levels at different time intervals

Percentage decrease in blood glucose concentration (mean ± sem)				
Time in hr	Pioglitazone (10mg/kg, p.o.)	Itraconazole (9mg/kg, p.o,7days) + Pioglitazone (10mg/kg, p.o.)	Pioglitazone (10mg/kg, p.o.)	Itraconazole (18mg/kg, p.o,7days) + Pioglitazone (10mg/kg, p.o.)
Fasting	-	-	-	-
1.0	15.91 ± 2.05	20.08 ± 2.17	11.67 ± 2.11	19.81 ± 1.34*
2.0	25.34 ± 1.52	22.80 ± 1.95	28.55 ± 2.16	30.05 ± 2.38
4.0	33.76 ± 1.81	42.80 ± 1.35*	35.02 ± 1.23	42.71 ± 2.65*
8.0	36.08 ± 1.57	38.63 ± 2.36	32.32 ± 3.73	42.46 ± 1.54**
12.0	26.09 ± 1.64	35.99 ± 2.54**	18.37 ± 2.01	38.65 ± 2.47***
18.0	18.21 ± 1.54	35.67 ± 2.01***	16.35 ± 2.15	40.85 ± 2.59***
24.0	10.50 ± 1.51	31.51 ± 1.08***	10.61 ± 1.37	39.76 ± 2.51***

Discussion

The present study indicated that drug interactions occur when itraconazole and pioglitazone are administered simultaneously at therapeutic doses. However, the therapeutic dose was found to influence the hypoglycemic effect significantly probably by inhibiting the CYP450 enzyme system. For the assessment of the potentiation of hypoglycemic effect, onset of action, (time taken to reduce minimum of 20% reduction in blood glucose levels), peak effect, duration of hypoglycemic effect (duration in which minimum of 20% reduction in blood glucose levels are maintained) were considered. Since itraconazole (18 mg/kg) perse did not influence the blood glucose levels and thus the possibility of occurrence of pharmacokinetic interaction can be ruled out. In our study, pretreatment with itraconazole (9 and 18mg/kg, p.o) altered the onset of action, peak effect and duration of hypoglycemic effect. Whereas higher dose, itraconazole (18mg/kg, p.o) has significantly enhanced duration of hypoglycemic effects distinguished with the lower dose of itraconazole (9mg/kg, p.o). This indicates that itraconazole retards the metabolism of these hypoglycemic drugs by inhibiting the enzymes responsible for their metabolism. There are reports that both pioglitazone and rosiglitazone are mainly metabolized by CYP2C8, CYP2C9 and CYP3A4.[18-20] Reports also indicate that itraconazole is a weak inhibitor of CYP1A2, CYP3A4, CYP2C9, CYP2C19 and CYP2D6.[20] The results have shown that the therapeutic dose of itraconazole enhanced the hypoglycemic effect of pioglitazone in healthy rats. Our studies in healthy rats suggested that pharmacokinetic drug interaction occurs between itraconazole and pioglitazone when they used simultaneously**.**

Conclusion

The present study clearly indicates that itraconazole has significantly enhanced the therapeutic efficacy of pioglitazone in healthy rats possibly inhibiting the CYP450 enzyme system. While administered, itraconazole and pioglitazone simultaneously precaution should therefore be taken to avoid the severe hypoglycemic effects. So therapeutic drug monitoring is required to adjust the appropriate dose of these concomitantly administered drugs.

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