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Research Article

PHARMACODYNAMIC AND PHARMACOKINETIC INTERACTION BETWEEN EFAVIRENZ AND PIOGLITAZONE- A STUDY IN ANIMAL MODELS

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ABSTRACT

The study was conducted in rats and rabbits with selected oral doses of Pioglitazone and Efavirenz their combination to evaluate the safety of Pioglitazone therapy in the presence of Efavirenz. Blood samples were collected from rats/rabbits by retro orbital/marginal ear vein puncture respectively at regular intervals of time. The blood glucose was estimated by GOD/POD method and serum Pioglitazone levels by HPLC method. Efavirenz showed to reduced blood glucose levels alone and with combination of Pioglitazone in both normal rats and diabetic rats. The serum Pioglitazone levels were increased significantly and pharmacokinetic parameters of Pioglitazone were altered significantly in presence of Efavirenz. The increased in the pharmacokinetic parameters like AUC, Cmax, Ka, Cl and increase in the $T_{1/2}$ indicate displacement of Pioglitazone from protein binding sites in the presence of Efavirenz. In conclusion, the combination might not be safe with respect to its hypoglycemic effect; care should be taken when Pioglitazone is administered with multiple doses of Efavirenz in a clinical situation.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia caused by defective insulin synthesis (type I), secretion (type II) coupled with resistance to insulin action or their combination.

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Diabetes prevalence is estimated to more than double by 2050. Both HIV infection and use of antiretroviral medications to treat HIV may be risk factors for diabetes [1,2]. This cross-sectional study of HIV-infected patients with diabetes mellitus found a prevalence of inadequate glycemic control of 33%. Associations with inadequate glycemic control included a more recent diagnosis of HIV, use of insulin or any diabetes medication, and higher triglyceride levels. Highly active antiretroviral therapy (HAART) in HIV infection produces a spectrum of metabolic complications, includ-

ing dyslipidemia, insulin resistance, and changes in body fat compartmentalization (peripheral lipoatrophy and central fat accumulation).[3] Thiazolidinediones (TZDs) have been introduced in the treatment of type 2 diabetes mellitus (T2DM) since the late 1990s. Although troglitazone was withdrawn from the market a few years later due to liver toxicity, both rosiglitazone and pioglitazone gained widespread use for T2DM treatment. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) Efavirenz, is now commonly for the treatment of human immunodeficiency virus (HIV) infections. Hence in the present study focusing on CYP3A4 inhibitors like non nucleoside reverse transcriptase inhibitors Efavirenz can be coadministered with a potent thiazolidinedione Pioglitazone to study the drug-drug interactions.

MATERIALS AND METHODS

Albino rats of either sex obtained from M/s. Mahaveer Enterprises, Hyderabad and albino rabbits of either sex obtained from M/s. Ghosh Enterprises, Kolkata were used in the study. All animals were maintained on pellet diet supplied by M/s. Rayan Biotechnologies Pvt. Ltd., Hyderabad with 12h/12h light/dark cycle and water ad libitum. Animals were fasted for 18 h before the experiment.

Study in normal rats

A group of six albino rats weighing between 250-300 g were administered with 10mg/ kg body weight Pioglitazone, orally. The same group was administered with 54mg/ kg body weight Efavirenz, orally after a wash out period of one week. The same group was also administered with 54 mg/ kg body weight Efavirenz 30 min prior to 10mg/ kg body weight Pioglitazone, after a further wash out period of 1 week. Blood samples were withdrawn from retro orbital puncture at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h intervals. Blood samples were analyzed for blood glucose levels by GOD/POD method [4] using commercial glucose kits (Span diagnostics).

Study in diabetic rats

Diabetes was induced by the administration of alloxan monohydrate in two doses 100 mg and 50mg/ kg body weight intraperitoneally for two consecutive days [5]. A group of 6 rats with blood glucose levels above 250 mg/dL was selected for the study. The study similar to the one conducted in normal rats was repeated in diabetic group.

Study in normal rabbits

A group of four albino rabbits weighing between 1.38-1.7 kg was used in the study. They were administered with 10mg/1.5 kg body weight Pioglitazone orally. The same group was administered with 42 mg/1.5 kg body weight Efavirenz given orally after a wash out period of 1 week. The same group was also administered with 42 mg/1.5 kg body weight Efavirenz (single and multiple dose treatment) 30 min prior to 10 mg/1.5 kg weight Pioglitazone was administered. Blood samples were collected at 0, 1, 2, 3, 4, 8, 12, 16 and 24 h intervals by puncturing the marginal ear vein in all experiments. Blood samples were analyzed for blood glucose levels by GOD/POD method using commercial glucose kits and for serum Pioglitazone concentration by HPLC method. The animal experiments were approved by our Institutional Animal Ethics committee and by the Government regulatory body for animal research (Regd. No. 1358/ERe/S/10/CPCSEA).

RESULTS & DISCUSSION:

Chronic diabetes mellitus precipitates other disorders in the long run leading to existence of several disorders simultaneously [6]. Such situations (existence of simultaneous multiple disorders) demand the use of more than one drug simultaneously known as polypharmacy which may precipitate drug interaction problems. In diabetic condition, maintenance of optimal level of blood glucose is essential since hyperglycaemia and hypoglycaemia are unwanted phenomenon. This is obtained by the use of antidiabetic drugs, proper diet and exercise [7]. Pioglitazone is a drug of choice in the type 2 diabetic patients since the situation of anti-diabetic drugs are used in combination therapy for longer period of time, whose safety and efficacy of drugs is in determine [8]. Pioglitazone is mainly metabolized in the liver by CYP2C9, CYP 2D6, CYP 3A4 and CYP 1A2 isozymes and is highly protein bound drug bound to proteins about 99% [9]. The drugs metabolized by the above enzymes are that inhibit/induce the above enzymes may interact with Pioglitazone when they are coadministered. Similarly, drugs with high protein binding nature may displace Pioglitazone from protein binding site and enhance the levels of Pioglitazone in the blood when they are coadministered. The oral administration of Pioglitazone produced a dose

dependant decrease in blood glucose levels in normal rats. The doses of selected drugs for interaction study were also fixed by extrapolating human therapeutic dose of selected drugs to rats and rabbits basing on body surface area [10]. The present study was planned to find out the influence of Efavirenz on pharmacodynamics of Pioglitazone in rats (rodent) and pharmacodynamics as well as pharmacokinetics of Pioglitazone in rabbits (non-rodent). Since rat and rabbit are two dissimilar species, if the interaction occurs in both the species, then there is more probability of its occurrence in humans also. Similarly, if absence of interaction is seen in both species, it is assumed to be absent in humans also. The normal rats were selected for preliminary and quick screening of the drugs and small volumes of blood were collected at regular time intervals for the estimation of blood glucose levels. Dose dependent relationship was observed with 5mg/kg, 10 mg/Kg, and 20mg/Kg body weight of Pioglitazone in normal rats. From these three doses 10 mg/Kg of Pioglitazone was selected for interaction study as it produced optimum blood glucose reduction, which is about 20-30% (graph 1). A dose of 10mg/kg body weight of Pioglitazone produced a steep reduction in blood glucose levels. The effect of Pioglitazone on blood glucose levels was studied in the absence and presence of the Efavirenz. In normal rats 54mg/kg body weight of Efavirenz produced hypoglycemic activity with peak activity at 2h and slightly increased the hypoglycemic effect of Pioglitazone when administered in combination (table 1). Based on the results obtained from the normal rats, the study was extended to alloxan induced diabetic rats to find out the drug interaction in diabetic condition. The selected dose of Pioglitazone (10 mg/kg bd.wt) was found to produce significant antihyperglycemic effect at 2 hr interval in diabetic rats. The activity produced with selected dose of Pioglitazone might be due to its mechanism of action for improving insulin sensitivity [11]. The drug solution of selected dose of Efavirenz (54mg/kg bd.wt) produced antihyperglycaemic activity with peak activity at 3 hr when administered alone and was found to enhance the antihyperglycaemic activity of Pioglitazone during 2 hr to 12 hr in combination. In combination, Efavirenz found to enhance the hypoglycemia produced by Pioglitazone in single dose treatment at 1 hr and 12 hr intervals (table 2). The antihyperglycemic activity of Efavirenz might be due to improve insulin resistance or have negligible alterations in glucose metabolism and are better choices [12].

In normal rabbits selected dose of Efavirenz showed slight reduction in blood glucose levels, which is not significant statistically, When administered in combination the selected dose of Efavirenz slightly enhanced the hypoglycemic activity of Pioglitazone at 1hr to 24hr intervals. The multiple dose treatment of Efavirenz for 7 days significantly enhanced hypoglycemic activity of Pioglitazone from 1hr to 24hr than single dose treatment (table 3). The serum insulin levels were found to be slightly altered with single dose treatment of Efavirenz, where as they are found to be increased significantly with multiple dose treatment of Efavirenz corresponding to the decreased glucose levels (table 4). The serum Pioglitazone levels were found to be slightly altered significantly with single dose treatment of Efavirenz (graph 2). There is a significant changes in the pharmacokinetic parameters like Cmax, T1/2, Vdss, Cl, AUC (0-α), AUMC (0-t), AUMC (0-α) and MRT of Pioglitazone with single dose treatment of Efavirenz and there is a significant change in Vdss in multiple dose of Efavirenz, this indicates there is a displacement of Pioglitazone from protein binding sites in the presence of Efavirenz (table 5). This may be because of high protein binding nature of Efavirenz[13].

Hence, the present study confirms that there is a combined pharmacodynamic interaction and pharmacokinetic interaction with single and multiple dose treatment of Efavirenz. Therefore, care must be taken to adjust the doses of Pioglitazone or advised to not safe when co-administered in a clinical situation.

CONCLUSION:

There was interaction in two dissimilar species (rats/rabbits), the combination of Efavirenz and Pioglitazone might not be safe in humans. Health care professionals should caution diabetic patients when such combination is prescribed. Serum glucose levels may need to be monitored by a healthcare provider, and medication adjustments may be necessary.

Graph 1: Comparison of % blood glucose reduction of Pioglitazone 5mg/kg, 10mg/kg and 20mg/kg.

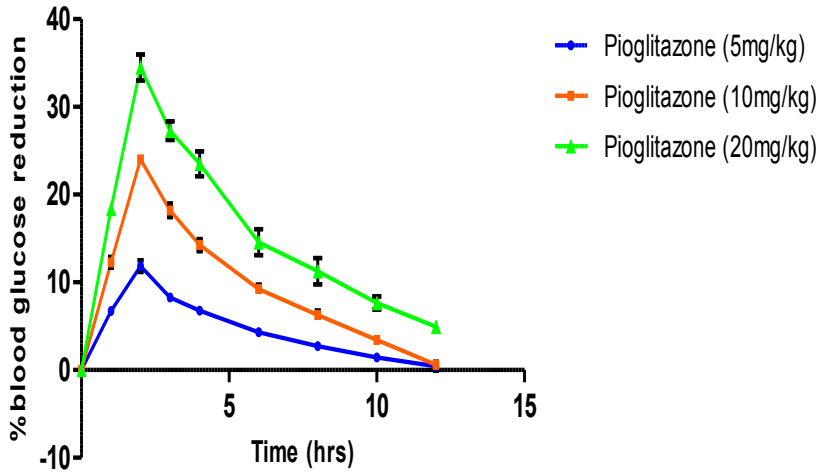

Table 1: Comparison of % blood glucose reduction of Pioglitazone 10mg/kg, Efavirenz (54mg/kg) and their combination in normal rats

Time (hrs)	% blood glucose reduction		
	Pioglitazone (10mg/kg)	Efavirenz (54mg/kg)	Combination
0	0.00±0.00	0.00±0.00 ^{ns}	0.00±0.00 ^{ns}
1	12.84±0.56	3.84±0.50***	16.12±0.86*
2	**36.22±12.38**	**6.64±0.46****	**38.70±0.88****
3	18.81±0.85	5.35±0.45***	32.46±0.98***
4	14.74±0.65	4.95±0.28***	26.99±1.13***
6	9.87±0.63	3.85±0.35***	23.64±0.92***
8	7.05±0.54	3.42±0.28***	18.60±0.84***
10	4.28±0.46	2.32±0.55 ^{ns}	15.28±0.74***
12	1.51±0.25	1.26±0.64 ^{ns}	11.32±1.01***

p<0.05*, p<0.01**, p<0.001*** Significance followed by one way ANOVA followed by Dunnet's multiple comparison test when compared with Pioglitazone (10mg/kg) group

Table 2: Comparison of % blood glucose reduction of Pioglitazone 10mg/kg, Efavirenz (54mg/kg) and their combination in diabetic rats

Time (Hrs)	% blood glucose reduction		
	Pioglitazone (10mg/kg)	Efavirenz (54mg/kg)	Combination
0	0.00±0.00	0.00±0.00 ^{ns}	0.00±0.00 ^{ns}
1	17.42±0.55	8.42±0.72***	28.06±1.30***
2	**31.94±0.66**	9.04±1.00***	**45.80±1.95****
3	25.89±1.17	**10.94±0.80****	39.72±2.42***
4	22.12±1.39	9.69±0.76***	35.58±2.62***
6	19.20±1.42	11.98±0.79*	32.05±2.84***
8	16.10±1.30	9.03±0.63*	26.75±4.53***
10	11.99±0.80	6.49±0.47 ^{ns}	25.67±2.00***
12	6.16±0.88	5.65±0.52 ^{ns}	23.63±1.71***

p<0.05*, p<0.01**, p<0.001*** Significance followed by one way ANOVA followed by Dunnet's multiple comparison test when compared with Pioglitazone (10mg/kg) group

Table 3: Comparison of % blood glucose reduction of Pioglitazone 10mg/kg, Efavirenz, Efavirenz + Pioglitazone (SD) and Efavirenz + Pioglitazone (MD)

Time (Hrs)	% blood glucose reduction			
	Pioglitazone (10mg/1.5kg)	Efavirenz	Efavirenz + pioglitazone (SD)	Efavirenz + pioglitazone (MD)
0	0.00±0.00	0.00±0.00	0.00±0.00 ns	0.00±0.00 ns
1	19.75±0.56	2.77±0.23	22.12±0.78 ns	25.31±0.56***
2	26.31±0.51	4.83±0.54	28.49±0.81 ns	35.18±0.74***
3	**35.26±0.35**	10.17±0.82	**39.35±1.03****	**47.65±0.74****
4	28.35±0.43	**12.98±0.68**	33.44±0.38***	43.95±0.95***
8	20.93±0.71	11.07±0.45	29.17±1.04***	38.40±0.64***
12	15.65±0.24	8.71±0.38	21.52±0.65***	34.48±0.60***
16	12.25±0.34	5.91±0.57	17.26±0.66***	31.57±0.52***
24	8.25±0.52	2.95±0.30	13.70±0.37***	20.44±1.56***

p<0.05*, p<0.01**, p<0.001*** Significance followed by one way ANOVA followed by Dunnet's multiple comparison test when compared with Pioglitazone (10mg/kg) group.

Table 4: Mean Serum insulin (µIU/mL) with mean serum glucose level (mg/dL) in Pioglitazone, Efavirenz and single and multiple dose treatment Efavirenz +Pioglitazone

Groups	Time (hrs)	Mean serum glucose levels (mg/dL)	Serum Insulin (µIU/mL)
Pioglitazone	0.00	101.00±4.16	9.62±0.56
	3.00	65.40±2.82	10.23±0.81
Efavirenz	4.00	81.60±2.64	10.12±1.06
Efavirenz + Pioglitazone (SD)	3.00	57.60±1.94	14.91±0.78
Efavirenz + Pioglitazone (MD)	2.00	51.00±1.95	15.55±0.59

Graph 2: The pharmacokinetic profile of Pioglitazone concentration Vs time with Pioglitazone formulation alone and its combination with Efavirenz in normal rabbits.

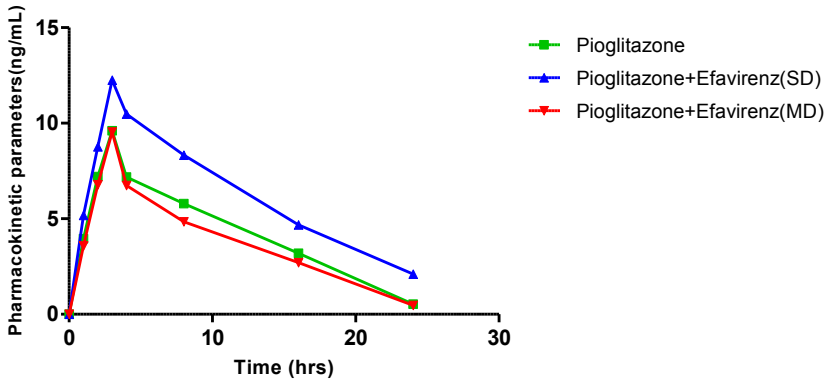

Table 5: Pharmacokinetic parameters of Pioglitazone with Pioglitazone alone and in combinations with Efavirenz in normal rabbits

Pharmacokinetic parameters	Pioglitazone	Efavirenz+Pioglitazone (SD)	Efavirenz+Pioglitazone (MD)
AUC0-24	101.24±0.3119	148.1±1.048	94.449±3.627
AUC 0-inf	105.64±0.4047	174.3±0.9883	92.532±0.8863
AUMC0-24	800.98± 4.7343	1586.8±20.679*	822.64±91.652
AUMC0-inf	947.59±7.39	2536.8±23.503**	784.97±16.809
Ke	0.1248±0.0003	0.078±0.002	0.1314±0.0012
Ka	1.536±0	1.536±0	1.536±0
T1/2	5.546±0.0103	8.59±0.0404	5.268±0.0434
Vdss	143788±31581	179375±1054.5**	191147±930.11***
Cl	21281±78.53	36037±23122*	19821±983.15
Tmax	3±0	3±0	3±0
Cmax	9.604±0.0427	12.258±0.0307	9.548±0.0712
MRT	8.8844±0.0201	14.553±0.0762	8.58±0.0991

$p<0.05$*, $p<0.01$**, $p<0.001$*** Significance followed by one-way ANOVA followed by Dunnet's multiple comparison test when compared with Pioglitazone (10mg/kg) group.

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