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# INFLUENCE OF VERAPAMIL ON PHARMACOKINETICS OF PIOGLITAZONE IN NORMAL AND DIABETIC RATS

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**ABSTRACT**: The study was undertaken to find out the pharmacokinetic drug interaction of verapamil on pioglitazone in normal and alloxan induced diabetic rats following single and multiple dose treatment. Human oral therapeutic doses of pioglitazone and verapamil were extrapolated to rats based on their body surface area. The serum pioglitazone concentrations were estimated by a sensitive reverse phase – high performance liquid chromatography (RP-HPLC) method. In single and multiple dose study, verapamil significantly increased the pioglitazone exposure (AUC, $C_{max}$) and decreased its elimination by prolongation of $t_{1/2}$ and mean residence time (MRT) with a relative decrease in clearance (CL). The effect is more significant in diabetic rats. In the present study a pharmacokinetic interaction between verapamil and pioglitazone was observed. The possible interaction may involve both P-glycoprotein and CYP enzymes. Investigating this type of interactions pre-clinically is helpful to avoid drug-drug interactions in actual clinical situation.

**INTRODUCTION:** Diabetes mellitus (DM) is a chronic metabolic disorder characterized by marked increase in blood glucose levels with altered metabolism of carbohydrate, protein and fat resulting from defective insulin secretion, resistance to insulin action, or a combination of both factors [1]. Treatment is given for prolonged periods, and may generally lasts lifelong. Incidence and prevalence of diabetes mellitus is ever increasing and is currently becoming a major threat to the world [2].

According to World Health Organization (WHO) estimate, there are 177 million people worldwide suffering from diabetes and this figure is likely to be more than double by 2030 [3]. Patient with diabetes mellitus may be associated with multiple disorders such as hypertension, dyslipidemias, arrhythmias, angina pectoris, fungal infections, and chronic elevation of glucose levels in the long run may precipitate several disorders like diabetic neuropathy, retinopathy, foot ulcers, etc., [4]. Polypharmacy which involves use of more than one drug for treating chronic disorders like diabetes mellitus or to treat multiple disorders which co-exist simultaneously in that patient is very common in present day clinical practice which may precipitate drug interaction problems. The drug treatment given to the patient is expected to improve the condition of the patient and should not lead to further deterioration.

Hence, care should be taken to avoid the possibility for over medication / under medication / unwanted effects of the combinations particularly used in clinical disorders. With oral hypoglycemic drugs, the addition of drugs used to treat hypertension is necessary in these patients. In such a situation, there may be chances for drug-drug interaction between these drugs.

Pioglitazone is a thiozolidinedione oral hypoglycemic compound commonly used in the treatment of type II diabetes. It is an insulin sensitizer that selectively stimulates the nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α [5]. From the *in vitro* data, it is found that pioglitazone metabolism is mediated through several cytochrome P450 (CYP) enzymes, predominant ones being CYP2C8 (40%) and to a lesser extent CYP3A4 (less than 20%) [6].

On the other hand, verapamil, a widely used calcium channel blocker for the treatment of various cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmias and coronary artery disease [7] is a substrate and inhibitor of p-glycoprotein and cytochrome P450 3A respectively [8-11]. Available literature data on verapamil reveals that *S*- and *R*-enantiomers of verapamil and norverapamil inhibits the metabolism of several coadministered CYP3A substrates by its capability in forming a metabolic intermediate complex (MIC) thereby resulting in clinically significant drug interactions [12].

The concomitant administration of pioglitazone with verapamil in diabetic patients suffering from hypertension may result in drug-drug interaction with enhanced/decreased pioglitazone activity, which is unwanted. The study is planned to establish the safety of the drug combination in animal models and find out the possible mechanisms responsible for the interaction if any.

## MATERIALS AND METHODS:

**Drugs and Chemicals:** Pioglitazone and rosiglitazone are the gift samples from Dr.Reddy's Lab (Hyderabad, India). Verapamil is obtained as gift sample from Aurobindo pharmaceuticals (Hyderabad, India). Alloxan monohydrate and HPLC grade methanol of S.D.Fine-Chemicals, Mumbai were procured from local chemical suppliers. All other chemicals and reagents used were of analytical grade.

**Animals:** Experiments were performed with Wistar rats of either sex weighing between 180 to 210gms procured from Mahaveer Enterprises (Approved by CPSCEA), Hyderabad, India. The animals were housed in colony cages (four per cage) under conditions of standard lighting of (12:12) hr light and dark cycle, temperature (22±1$^{o}$C) and relative humidity for at least one week before beginning of experiment, to acclimatize to the new environment and to overcome stress possibly incurred during transit. During this period, they had free access to food and water. A fast of at least 18 hours is maintained prior to the each experiment and was withdrawn from food and water during the experiment. The experiments were planned and conducted after prior approval of Institutional Animal Ethical Committee (IEAC), University College of Pharmaceutical Sciences, Warangal, A.P., India.

**Selection of doses in the Study:** In clinical practice, pioglitazone and verapamil were administered orally in therapeutic dose as antidiabetic and antihypertensive drugs respectively. Oral therapeutic dose in humans for pioglitazone and verapamil were extrapolated to rats based on body surface area [13] and were used in the experiment.

**Single dose and Multiple dose Pharmacokinetic study in normal healthy rats:** Wistar rats of either sex were randomly distributed into three groups of six animals each. Prior to experiment, the rats were fasted for 18 hours (hrs) and water *ad libitum*. Water was withdrawn during experiment. After collection of predose (0.0 hr) blood samples, drugs were administered orally according to their body weight (b.wt) in the following order.

Group I — Pioglitazone (10 mg/kg) [14].

Group II— Pretreated with verapamil (9 mg/kg) [15] followed by pioglitazone (10 mg/kg) after 30 minutes.

Group III — Pretreated with verapamil (9 mg/kg) once daily for 7 days, and on 8th day after overnight fasting of at least 14 hrs, verapamil (9 mg/kg) is administered followed by Pioglitazone (10 mg/kg) after 30 minutes.

**Single dose and Multiple dose Pharmacokinetic study in Diabetic rats:**

**Induction of Diabetes** [16]: Experimental diabetes in rats was induced in 18 hrs fasted rats by intraperitoneal (i.p) injection of aqueous solution of alloxan monohydrate dissolved in sterile saline in two doses i.e. 100 mg and 29 mg/kg body weight, for two consecutive days. At 72 hrs, the blood samples from all surviving rats were estimated for glucose levels.

Rats with blood glucose levels of 250 mg/dL and above were considered as diabetic and selected for the study. Care is exercised throughout the induction period to prevent occurrence of sudden hypoglycemic states by intermittent feeding with 10% dextrose solution and standard pellet diet. The same treatment as mentioned in single dose and multiple dose pharmacokinetic experiment in normal rats is repeated in a similar fashion, but in alloxan induced diabetic rats.

**Collection of Blood Samples**: Blood was collected from retro orbital plexus [17] using heparinized capillaries into micro centrifugation tubes at 0 (predose), 0.5, 1, 2, 4, 6, 8, 12 and 24 hour time intervals after each treatment. Serum was separated by centrifugation and stored in vials at −70°C until further analysis.

**Bioanalytical method** [18]: Pioglitazone concentrations in serum were determined using a validated RP-HPLC method. Briefly, the HPLC system consisted of a Waters 717 plus autosampler (Waters Co, Milford, Massachusetts), a Waters 501 pump (Waters Co), and a 785 UV detector (Applied Biosystems, Foster City, California) operated at 247nm. The stationary phase was a Waters Symmetry C18 column (250 × 4.6 mm, 5 µm, Waters Co). The mobile phase consists of ammonium acetate (30mM; pH 5), methanol (65:35 v/v) at a flow rate of 1.0 mL/min.

Pioglitazone and an internal standard (rosiglitazone) were isolated from serum by liquid-liquid extraction with ethyl acetate. The organic phase was separated and evaporated, and the remaining residue was reconstituted with 250 µL of mobile phase before application on to the HPLC system. The method was validated and found to be linear over the concentration range of 0.1 to 10.0 µg/mL. Using a linear weighted least squares regression, the lower limit of quantitation (LLOQ) was 0.1µg/mL. The intra-assay and interassay coefficients of variation (%CV) for the 4 quality control standards (0.25, 2.00, 8.00, and 30.0 µg/mL) were ≤10.2% and 4.9%, respectively. The accuracy ranged between 96.7% and 99.1% for the serum samples.

**Pharmacokinetic analysis:** The pharmacokinetic parameters of pioglitazone were computed using a sophisticated tool known as WinNonlin, Version 4.1 (Pharsight Corporation, USA) and the parameters includes area under the curve (AUC), peak exposure ($C_{max}$), time to peak exposure ($t_{max}$), half-life, ($t_{1/2}$), Clearance (CL), and mean residual time (MRT).

**Statistical analysis:** The data was expressed as mean ± standard deviation (SD). The significance was determined by applying one way ANOVA. A value of $P < 0.05$ was considered statistically significant.

**RESULTS:** In the present study, serum pioglitazone levels were increased and its pharmacokinetic parameters namely AUC, $C_{max}$, $t_{max}$ $t1_{/2}$, CL and MRT were altered significantly with single and multiple-dose administration with verapamil in normal rats. The pharmacokinetic parameters of pioglitazone alone, single dose treatment, SDT [verapamil (9 mg/kg b.wt) administered 30 minutes prior to pioglitazone (3.6mg/kg) treatment] and multiple dose treatment, MDT [verapamil once daily for consecutive 7 days and on 8$^{th}$ day verapamil (9 mg/kg b.wt ) followed by pioglitazone (10 mg/kg), 30 minutes later] in healthy rats were shown in **Table 1**. In single dose study with verapamil in normal rats, percentage increase in serum concentrations of pioglitazone in comparison with the pioglitazone alone treated rat group (Group-I) for the various pharmacokinetic parameters is AUC (3.1%), $C_{max}$ (4.3%), $t_{1/2}$ (4.7%), , MRT (13.7%) with a relative decrease in clearance (CL) by 6.5% and slight decrease in $t_{max}$ of 3.0%. In multiple dose study with verapamil, the percentage increases in pharmacokinetic parameters of pioglitazone are as follows: AUC (10.3%), %), $C_{max}$ (4.3%), $t_{1/2}$ (39.8%), and MRT (32.8%) with a relative decrease in CL by 12.7% and $t_{max}$ by 2.99.

In diabetic rats, single and multiple dose treatment with verapamil altered the serum concentrations and pharmacokinetic parameters of pioglitazone in a similar manner as that observed in normal rats but are statistically significant (**Table** 2). In single dose study, verapamil increased the serum concentrations of pioglitazone as reflected from the pharmacokinetic parameters, AUC (7.8%), $C_{max}$ (4.3%), $t_{1/2}$ (19.0%), with a relative decrease in MRT (1.4%), $t_{max}$ (4.2%) and CL (2.1%). In multiple dose study with verapamil, the percentage increases in pharmacokinetic parameters of pioglitazone are as follows: AUC (31.1%), $C_{max}$ (19.2%), $t_{1/2}$ (35.3%), and MRT (36.1%) with a relative decrease in CL by 7.6% and $t_{max}$ by 2.6%.

**TABLE 1: PHARMACOKINETIC PARAMETERS OF PIOGLITAZONE IN PRESENCE OF VERAPAMIL IN NORMAL RATS. (N=6)**

| PK Parameter | Pioglitazone | Pioglitazone + Verapamil (SDT) | Pioglitazone + Verapamil (MDT) |
|---|---|---|---|
| AUC (µg.h/mL) | 102.45±2.83 | 105.73±8.31 | 113.02±3.11** |
| $C_{max}$ | 11.69±0.52 | 12.22±0.67 | 14.47±1.44** |
| $t_{max}$ | 3.79±0.48 | 3.68±0.64 | 3.74±0.73 |
| $t_{1/2}$(h) | 3.47±0.59 | 4.71±1.00* | 4.85±0.28* |
| Cl (ml/min) | 340.44±20.90 | 344.34±30.17** | 311.43±36.3* |
| MRT | 8.84±1.12 | 10.24±1.693 | 11.74±0.44* |

*- Significant at P<0.05, **- Significant at P<0.01, compared to pioglitazone control, Values were represented as mean ± SD.
SDT – Single dose treatment with verapamil (9 mg/kg) administered 30 minutes prior to pioglitazone (10 mg/kg) treatment
MDT – Multiple dose treatment with verapamil once daily for 7 consecutive days and on 8th day verapamil (9 mg/kg) followed by pioglitazone (10 mg/kg) 30 minutes later

**TABLE 2: PHARMACOKINETIC PARAMETERS OF PIOGLITAZONE IN PRESENCE OF VERAPAMIL IN ALLOXAN INDUCED DIABETIC RATS**

| PK Parameter | Pioglitazone | Pioglitazone + Verapamil (SDT) | Pioglitazone + Verapamil (MDT) |
|---|---|---|---|
| $AUC_{0-\infty}$ (µg.h/mL) | 115.23±3.57 | 124.22±4.42* | 151.07±2.75** |
| $C_{max}$ (µg/mL) | 11.69±0.52 | 12.22±0.67 | 14.47±1.44** |
| $t_{max}$ | 3.84±0.75 | 3.68±0.64 | 3.74±0.73 |
| $t_{1/2}$(h) | 3.99±0.98 | 4.75±2.39* | 5.20±2.17** |
| CL (ml/min) | 316.94±68.34 | 310.28±115.87* | 292.85±107.35** |
| MRT(h) | 9.850±1.66 | 9.712±3.95 | 13.41±3.63* |

* p<0.05; ** p < 0.01. Values were represented as mean ± SD.
SDT – Single dose treatment with verapamil (9 mg/kg) administered 30 minutes prior to pioglitazone (10 mg/kg) treatment
MDT – Multiple dose treatment with verapamil once daily for 7 consecutive days and on 8th day verapamil (9 mg/kg) followed by pioglitazone (10 mg/kg) 30 minutes later

**DISCUSSION:** Drug interactions are observed in clinical practice, but the mechanism of such interaction need to be explored in animal models [19] prior to its application in humans to ensure safety of drug combination. Hence animal models were used in understanding the possible mechanisms of such interactions. We studied the influence of verapamil on the pharmacokinetics of pioglitazone at therapeutic doses in healthy and alloxan induced diabetic rats. The healthy rat model served to quickly identify the interaction and the diabetic rat model served to validate the same response in the actually used diseased condition [20].

The serum concentrations of pioglitazone were increased in normal and diabetic rats upon single dose administration with verapamil as indicated from its pharmacokinetic parameters (AUC, $C_{max}$, $t_{max}$, $t_{1/2}$, CL and MRT). A more significant increase in serum concentrations of pioglitazone is observed in normal and diabetic rats on multiple dose treatment with verapamil. This increased bioavailability (AUC and $C_{max}$) of pioglitazone when taken with verapamil led to increased circulating levels of pioglitazone and its slower elimination (as reflected from its clearance and half life) may results in enhanced blood glucose lowering activity which may further precipitate hypoglycemic toxicity.

This increased bioavailability and decreased elimination of pioglitazone when coadministered with verapamil in normal and diabetic rats might be a result of inhibition of p-gp and/or its CYP 3A mediated metabolism. Also studies on diabetic rats, revealed that there is reduced CYP 3A activity [21] as well as reduced function of P-gp transporters in diabetic state [22], a close resemblance of the condition observed in diabetic patients. Published literature on the effect of various inhibitors on pioglitazone pharmacokinetics in humans found to exhibit a weak *in vivo* inhibitory effect. In a study carried out by Prasad *et al*, it is observed that pioglitazone lack the regeneration capacity of P-gp in diabetic rats [23].

Hence, the possible mechanism of increased bioavailability of pioglitazone when coadministered with verapamil might be the combined effect of decreased P-gp function and inhibition of P-glycoprotein mediated transport in gastrointestinal tract and in renal tubules as well as inhibition of CYP3A mediated metabolism.

**CONCLUSION:** The interaction observed appears to be pharmacokinetic at absorption, metabolic and excretion levels. Hence, the combination of pioglitazone and verapamil should be contraindicated or used with caution in a clinical situation.

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