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Effects of ritonavir on the pharmacokinetics and pharmacodynamics of pioglitazone in normal and diabetic rats

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ABSTRACT

The aim of the study is whether the impact of ritonavir will increase the plasma level of pioglitazone or not. Ritonavir is an antiretroviral drug to treat HIV AIDS and inhibits cytochrome P450-3A4. Multiple CYP isoforms are involved in the metabolism of pioglitazone like CYP2C8 and CYP3A4. Hence there is more possibility of ritonavir to inhibit the metabolism of pioglitazone by inhibiting CYP 3A4. Ritonavir (10 mg/kg,p.o.) alone and alongwith pioglitazone (10mg/kg, p.o.) was given to normal and diabetic rats. PK/PD parameters were studied. In the rats co-treated with ritonavir and pioglitazone, fasting plasma glucose concentration (58.93±3.20%) was further reduced. The pharmacokinetic parameters like clearance (12.20±0.625 l/hr) of pioglitazone was reduced, peak plasma concentration (16.78±1.08ug/ml), area under the plasma concentration time curve (163.5±14.82ug/ml/hr) and elimination half-life (6.194±0.81hr) were significantly increased when compared to pioglitazone treated rats.This study revealed that ritonavir affected the disposition of pioglitazone in rats probably by the inhibition of CYP3A4, leading to increasing pioglitazone concentrations that could increase the efficacy of pioglitazone or it may causes severe hypoglycemia. Therefore, its warrants to use relatively less dose of pioglitazone than the normal dose required for the beneficial effect on hyperlipidamia induced by ritonavir.

Key words: Cytochrome P-450 3A4, Metabolism, drug-drug interactions, Diabetes Mellitus, Pharmacokinetics & Pharmacodynamics.

INTRODUCTION

Pioglitazone is a thiazolidinedione derivative antidiabetic agent that acts primarily by decreasing hepatic and peripheral insulin resistance via its action at the peroxisome proliferator activated receptor subtype gamma (PPAR-γ) [1,2] and improving hyperglycemia and hyperlipidemia in type 2 diabetes mellitus.[3] This beneficial effect on hyperlipidemia has been well established in patients with diabetes. Pioglitazone has significantly reduced the elevated levels of serum triglycerides and cholesterol in both atherogenic diet and triton induced hyperlipidemia.[4] One study showed that non-HIV patients treated with pioglitazone had mean decreases in triglycerides, mean increases in high-density lipoprotein (HDL) cholesterol, and no consistent mean changes in LDL and total cholesterol.[5]

The pharmacokinetic studies indicate about 80% oral bioavailability of pioglitazone, and it is suggested that it is metabolized by multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites. A recent report suggests that rifampicin; an inducer of CYP3A4 decreased the area under the curve (AUC) of pioglitazone by 35% whereas, gemfirozil; an inhibitor of CYP2C8 increased the AUC by 239% .[6] These evidences suggest that pioglitazone can be a prime candidate for several drug–drug interactions, particularly because large number of drugs is reported to modulate the CYP450 enzyme activity.

Hyperlipidemia with increased total and low-density lipoprotein (LDL) cholesterol has been associated with coronary heart disease in the general population.[7] In human immunodeficiency virus (HIV)–infected individuals who are undergoing antiretroviral therapy (ART), lipid elevations have been reported at higher frequencies compared with the general population.[8,9] It has been reported that in the first 4 to 6 years of highly active antiretroviral therapy (HAART), there is a 25% increase likelihood of coronary artery disease.[10,11] Although it remains uncertain whether such metabolic changes can actually accelerate cardiovascular disease and how this might occur, physicians treating HIV-infected patients are nevertheless interested at lowering any potential cardiovascular risks in this patient population .[12] Several studies have shown that the rate of hyperlipidemia can be high especially in patients treated with protease inhibitor – based regimens.[13,14,15,16] Glitazones are useful to prevent the toxic effect of protease inhibitors on adipogenesis.[17] It is evident that an HIV-infected patient who developed hyperlipidemia 1 month after initiating a PI-boosted regimen (tipranavir / ritonavir), with significant improvement in total cholesterol and triglyceride levels using pioglitazone 60 mg daily.[18] Ritonavir is one of the antiretroviral drug from the protease inhibitor (PI) class used to treat HIV infection and AIDS. Ritonavir inhibits cytochrome P450-3A4 (CYP3A4) and is metabolized by CYP3A4 and CYP2D6 .[19,20,21,22] Among available protease inhibitors, ritonavir carries the highest risk of incurring drug interactions due to inhibition of cytochrome P450 activity.[23] In view of the influence of ritonavir on CYP3A activity, its presence in antiretroviral therapy may influence the pharmacokinetics of pioglitazone, particularly because pioglitazone is metabolized by CYP3A. Therefore, the aim of the present investigation was to study the influence of ritonavir on pharmacokinetics of pioglitazone. Female rats were treated with inhibitory dose of ritonavir, and its influence on the pharmacokinetics of orally administered pioglitazone was studied. The pharmacokinetics of pioglitazone is well characterized in rats along with the appreciation of gender differences. Hence, the present investigations were carried out using female Wistar rats as an animal model.

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2. MATERIALS& METHODS:

2.1. Materials

Pioglitazone, rosiglitazone and ritonovir are the gift samples from Dr Reddy Labs (Hyderabad, India). Alloxan monohydrate was purchased from Sigma Aldrich (MO, USA). Glucose kits of Span diagnostics were procured from local suppliers. The HPLC grade methanol and ammonium acetate (Merck, Mumbai, India). All other chemicals used were of analytical grade. The

drug analysis was carried out using HPLC system (Shimadzu 10AT/Vp, Kyoto, Japan) having Rheodyne injector port (20 µl loop), and UV/vis detector (SPD 10A Vp). The data interpretation was done with LC-solutions (Shimadzu, Kyoto, Japan) data acquisition software.

2.2. Animals:

Female Wister rats weighing between 180-230 g, procured from Mahaveerar Enterprises (Hyderabad, India) were used in the study. They were maintained under standard laboratory conditions at ambient temp of 25 ±2?C with 12-hour light/12-hour dark cycle. They were fed with standard pellet diet (Mahaveerar Enterprises Pvt. Ltd, Hyderabad, India) and water *ad libitum*. The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethics Committee (IAEC/05/UCPSC/KU/2010).

2.3. Study design

2.3.1. Pharmacokinetic and Pharmacodynamic interaction study in normal rats.

Rats were divided into 4 groups each for both normal and diabetic separately (n=6).

Group 1 -Pioglitazone (10 mg/kg;p.o) .[24]

Group2 -Ritonavir (10 mg/kg;p.o., 5% aqeous methyl cellulose as vehicle,control animals recived vehicle only) .[25]

Group 3 - Ritonavir (10 mg/kg;p.o.) followed by 1 hour after pioglitazone (10 mg/kg;p.o). (Single dose interaction; sdi)

Group 4 - Pretreated with ritonavir (10 mg/kg) for 7 days, on the 8 th day ritonavir (10 mg/kg;p.o.) followed by 1 hour after pioglitazone (10 mg/kg). (Multiple dose interaction; mdi)

Blood samples (0.5ml) were collected from via jugular vein at time intervals 0, 0.5, 1, 2, 4, 8, and 24 h postdose. Whole blood from donor rats was infused into each study rat to maintain blood volume lost due to sample collection (25). Serum was separated by centrifugation at 8000 rpm for 10 min using Biofuge-13 (Heraeus Instruments, Germany). And blood glucose levels were determined using Glucose Oxidase Peroxidase (GOD POD) method by measuring optical density spectrophotometrically at 510nm [26] and remaining serum was stored in vials at -20° C until further analysis.

2.3.2. Pharmacokinetic and Pharmacodynamic interaction study in diabetic rats.

Similarly a separate set of four groups (n=6), pharmacokinetic and pharmacodynamic interaction study explained above was repeated in diabetic rats.

Pioglitazone concentration was determined by slight modification of a method reported by (27,28). In brief, to 100 µl of plasma sample, 100 µl of rosiglitazone (10 µg in methanol) solution as internal standard added. The mixture was vortex mixed for 5 min after which it was centrifuged at 10,000 g for 10 min. Supernatant was collected and used for analysis. 20 µl of the supernatant was injected onto the HPLC system for analysis. The UV detector was set at 247 nm for the present analysis. C18 column (Grace Smart.250×4.6mm,5um) Luna, PHENOMENEX1, USA. The flow rate was 1.0 ml/min and the mobile phase consisted of 30 mM ammonium acetate (pH 5.0), and methanol in a ratio of 65:35 (v/v). The method was linear over 0.510–20 µg/ml.

2.4. Induction of Diabetes in rats.

Diabetes was induced in rats by the administration of alloxan monohydrate [29] in ice cold normal saline150 mg/kg bd wt intraperitoneally .[30,31,32] After 72hr, sample was collected from rats by via jugular 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.

2. 5. Glucose reduction calculations

Percentage Reduction in BGL = {(IBGL – FBGL)/ IBGL} × 100

where

BGL = blood glucose level;

IBGL = initial blood glucose level;

FBGL = final blood glucose level.

2.6. Data analysis

2.6.1. Pharmacokinetic data analysis

The maximum plasma concentration (C_{max}), time needed to reach the maximum plasma concentration (T_{max}), area under the concentration– time curve (AUC_{0-8}), mean residence time (MRT), elimination rate constant (K_{el}), clearence and half life ($T_{1/2}$) were calculated using non-compartmental pharmacokinetic model of WinNonlin-4.0.

2.6.2. Statistical analysis

All the means are presented with their standard deviation (mean ±S.D). The pharmacokinetic parameters of pioglitazone groups were compared using one-way ANOVA, followed by post hoc Dunnett test. An unpaired Student's t-test was used to determine the significant difference between the percentage glucose reduction values and pharmacokinetic parameters of pioglitazone in control and ritonavir treated groups. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Pharmacokinetic and Pharmacodynamic interactions in Normal Rats

Mean serum concentration of pioglitazone in absence and presence of ritonavir (sdi & mdi) in normal rats were shown in Fig. 1. Mean pharmacokinetic parameters of pioglitazone in presence of ritonavir in normal rats were shown in Table 1 and percentage reduction is shown in Fig 2. The pharmacokinetic parameters of pioglitazone like AUC_{0-8}, C_{max}, $T_{1/2}$, Clearance and MRT were altered significantly with single and multiple dose treatments of ritonavir in normal rats when compared to pioglitazone alone treated group. Pioglitazone produced hypoglycemic activity indicated by percentage glucose reduction levels in normal rats. Hypoglycemic effect of pioglitazone enhanced when given in combination with ritonavir it was indicated by significant increase in percentage glucose reduction in comparison to pioglitazone alone treated group in normal rats (Table 2, Fig. 2).

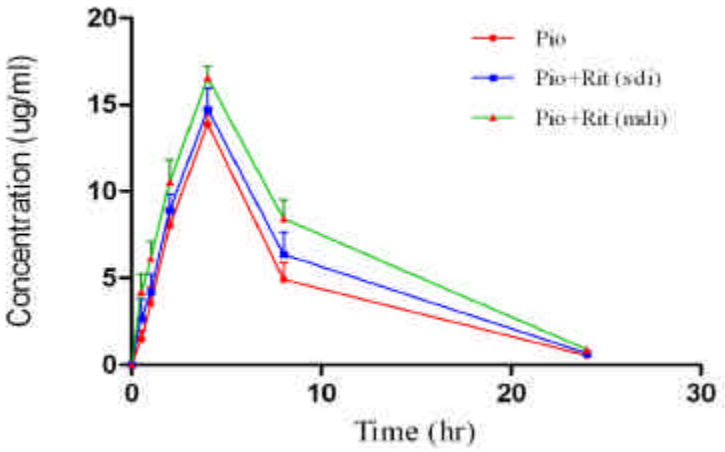

Fig.1. Plasma concentrations time curves of pioglitazone following its oral administration at 10 mg/kg in control and ritonavir (10 mg/kg) pre-treated (sdi & mdi) normal rats. Data are expressed as mean±S.D. in (n = 6) rats.

Table.1. Mean pharmacokinetic parameters of Pioglitazone alone and in presence of Ritonavir (sdi & mdi) in normal rats .AUC: area under the plasma concentration curve; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $T_{1/2}$: elimination/biological half life; MRT: mean residence time; Mean ±SD (n=6); $^{**}p<0.01$; $^{*}p<0.05$ compared to pioglitazone control; sdi (Single Dose Interaction); mdi (Multiple Dose Interaction), (two-tailed Student t-test).

Parameters	Pioglitazone	Pioglitazone +ritonavir(sdi)	Pioglitazone +ritonavir(mdi)
AUC_{0-8} (µg-h/ml)	112.00±73.38	135.90±14.42 *	167.40±11.78 **
C_{max} (µg /ml)	13.87±0.92	14.72±1.24	16.54±0.68 **
T_{max} (h)	3.9±0.23	3.7±0.54	3.8±0.65
Clearence (ml/h)	17.91±1.17	14.83±1.54 **	11.99±0.81 **
$T_{1/2}$ (h)	4.27±1.28	5.68±0.43*	6.12±0.25**
MRT (h)	7.70±0.68	8.80±0.31 *	8.91±0.67 *

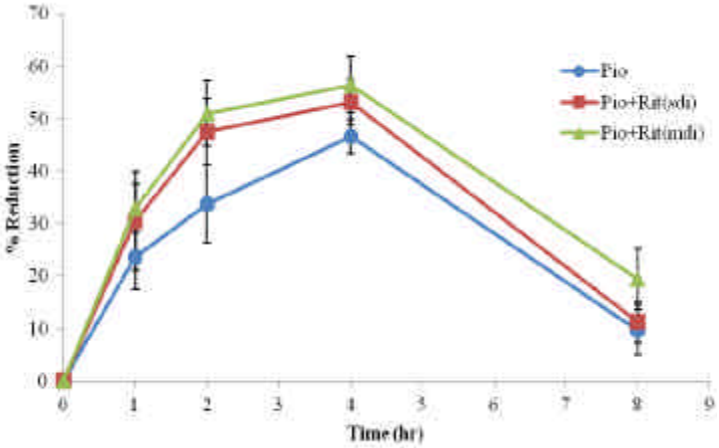

Fig.2. Mean percentage blood glucose reduction in normal rats after oral administration of only pioglitazone and pioglitazone with ritonavir (sdi & mdi). Data are expressed as mean±S.D. (n = 6).

3.2. Pharmacokinetic and Pharmacodynamic interactions in Diabetic Rats.

Mean serum concentration of pioglitazone in absence and presence of ritonavir (sdi & mdi) in diabetic rats were shown in Fig. 3. Mean pharmacokinetic parameters of pioglitazone in presence of ritonavir in diabetic rats were shown in Table 2 and percentage reduction is shown in Fig.4. The pharmacokinetic parameters of pioglitazone like AUC_{0-8}, C_{max}, $T_{1/2}$, and Clearance were altered significantly with single and multiple dose treatments of ritonavir in diabetic rats when compared to pioglitazone alone treated group. Pioglitazone produced hypoglycemic activity indicated by percentage glucose reduction levels in diabetic rats (Table 2). Hypoglycemic effect of pioglitazone enhanced when given in combination with ritonavir it was indicated by significant increase in percentage glucose reduction in comparison to pioglitazone alone treated group in diabetic rats (Table 2, Fig.4).

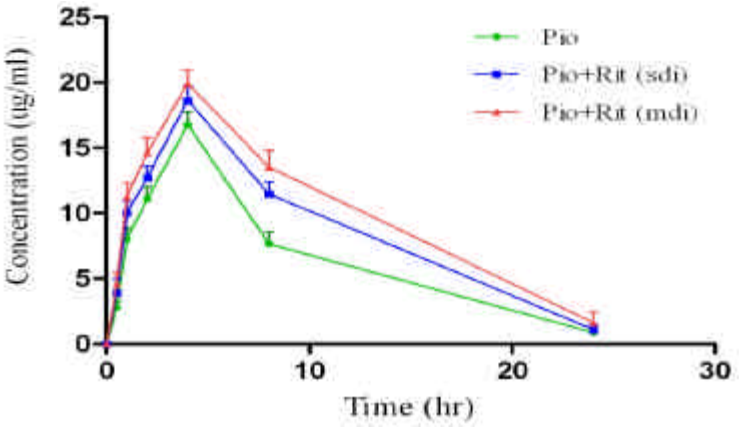

Fig.3. Plasma concentrations time curves of pioglitazone following its oral administration at 10 mg/kg in control and ritonavir (10 mg/kg) pre-treated (sdi & mdi) diabetic rats. Data are expressed as mean±S.D. in (n = 6) rats.

Table.2. Mean pharmacokinetic parameters of pioglitazone in presence of ritonavir (SDI & MDI) in diabetic rats. Mean ±SD (n=6); $^{**}p<0.01$; $^{*}p<0.05$ compared to pioglitazone control; sdi (Single Dose Interaction); mdi (Multiple Dose Interaction), (two-tailed Student t-test).

Parameters	Pioglitazone	Pioglitazone +ritonavir(sdi)	Pioglitazone +ritonavir(mdi)
$AUC_{(0-8)}$ (µg-h/ml)	128±25.25	163.5±14.82*	183.93±18.94**
C_{max} (µg/ml)	14.95±0.9843	16.78±1.085*	18.43±1.055**
T^{max} (h)	4.00±0.24	3.9±0.35	3.7±0.48
Clearence (ml/h)	15.074±0.9749	12.20±0.62569*	10.80±0.5868**
$T_{1/2}$ (h)	5.01±0.5215	5. 94±0.8198*	6.49±1.393**
MRT (h)	8.43±0.4202	8.792±0.894	8.88±1.529

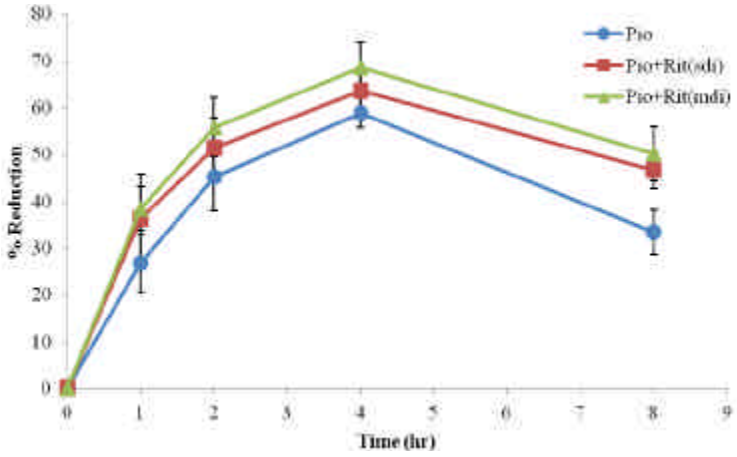

Fig.4. Mean percentage blood glucose reduction in diabetic rats after oral administration of only pioglitazone and pioglitazone with ritonavir (sdi & mdi). Data are expressed as mean±S.D.(n = 6).

4. DISCUSSION

Thiazolidinediones are used to treat type 2 diabetes especially with pioglitazone alone or combination with other hypoglycaemic agents, in both animal and human models, which act by enhancing peripheral sensitivity to insulin. Several theories are explaining about the weight gain associated with pioglitazone due to increased appetite,[33] increase in the amount of subcutaneous fat [34,35] and fluid retention.[35,36] Thiazolidinediones are potent antidiabetic compounds, which act by enhancing peripheral insulin sensitivity. It has been suggested that insulin resistance is involved in the impaired vascular endothelial function pioglitazone improves endothelial function in nondiabetic hypertensive individuals with insulin resistance, and that the improvement is associated with the amelioration of insulin resistance itself rather than that of hyperglycemia or hyperinsulinemia. [37] Left ventricular (LV) hypertrophy and diastolic dysfunction, which are common cardiac consequences of hypertension, are modified by insulin resistance. Beneficial effect of pioglitazone, due to the amelioration of insulin resistance on diastolic function as primary treatment of insulin resistance may reverse such cardiac changes in hypertensive patients.[38] Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker fa/fa rat and hence the capacity of adipose tissue to utilize glucose. Muscles are the major site for insulin-mediated glucose disposal, the increase of muscle glucose utilization under thiazolidinedione treatment could be secondary to local adipose tissue differentiation.[39]

Pioglitazone efficiently promotes adipocyte differentiation in vivo related to both an enhanced number of small adipocytes in the retroperitoneal fat pad, and a direct effect of pioglitazone on specific gene expression (phosphoenolpyruvate carboxykinase and ob genes) in mature adipocytes.[40] Glitazones are useful to prevent the toxic effect of protease inhibitors on adipogenesis *in vitro* by enhancing perixosome proliferator-activated receptor γ activity, it suggesting that this class of drug might be useful in the treatment of lipodystrophy in HIV-1-infected patients on HAART. [17] An HIV-infected patient who developed hyperlipidemia 1 month after initiating a PI-boosted regimen (tipranavir / ritonavir), with significant improvement in total cholesterol and triglyceride levels using pioglitazone.[18] This effect on lipids can be explained by pioglitazone's high affinity for intracellular ligands for peroxisome proliferator–activated receptors PPARs.[41,42] The PPAR is a family of ligand-activated transcription factors. Three subtypes have been identified: PPAR-α (predominantly in the liver), which regulates the expression of genes involved in the fatty acid catabolism that reflects in lower cholesterol and triglycerides; PPAR-γ, which is involved in adipocyte differentiation and lipid storage and PPAR-δ [43] by using transactivation assays, pioglitazone has been shown greater activity for PPAR-a may explain effect in lipids. [41,44] The activation of these systems is dose-depen

dent. This can explain why the changes in our patient's cholesterol and triglyceride levels did not occur at lower doses of pioglitazone.[44] Thiazolidinediones also increase the expression and plasma concentration of adiponectin, a protein secreted by the adipocytes, which play a role in the development of insulin resistance and dyslipidemia in HIV patients.[45] Although the risk of hypoglycemia with thiazolidinediones appears negligible but drug interactions may exacerbate adverse effects and raise safety concerns. [6] Several studies have reported that ritonavir is a potent inhibitor of CYP3A4, which may plays a role in the metabolism of pioglitazone. Accordingly, the present study was designed to clarify the effect of ritonavir on the CYP3A activity and, further to test the hypothesis that pioglitazone pharmacokinetics may be altered in the presence of ritonavir, as it is metabolized via CYP3A4 in humans.The pharmacokinetic parameters of pioglitazone like AUC_{0-8}, C_{max}, $T_{1/2}$, and Clearance were altered significantly with single and multiple dose treatments of ritonavir (100 mg/kg) in normal and in diabetic rats when compared to pioglitazone alone treated group. The increase in oral AUC_{0-8} can be attributed to a decline in hepatic clearance. Due to the increase in the plasma levels of pioglitazone by inhibition of CYP-mediated metabolism the elimination rate constant was decreased and $t_{1/2}$ was increased. It has been reported that ritonavir CYP3A inhibitory activity was substantiated with *in vivo* midazolam pharmacokinetics in dexamethasone pretreated female rats and *in vitro* erythromycin-N-demethylation assay (28). Increase in plasma concentration of pioglitazone was also indicated by enhanced percentage glucose reduction with single and multiple dose treatments of ritonavir 100 mg/kg in normal and in diabetic rats when compared to pioglitazone alone treated group.

Pioglitazone is a thiazolidinedione derivative antidiabetic agent that acts primarily by decreasing hepatic and peripheral insulin resistance, therefore improving hyperglycemia and hyperlipidemia in type 2 diabetes mellitus.[17] This beneficial effect on hyperlipidemia has been well established in patients with diabetes This effect on lipids can be explained by pioglitazone's high affinity for intracellular ligands for peroxisome proliferator-activated receptors (PPARs) [41] the effectiveness of pioglitazone 60 mg daily which is above the recommended maximum dose of 45 mg/d. It is unsure whether higher doses of pioglitazone may be required in some cases to achieve a beneficial effect on hyperlipidemia induced by ART. Small studies have not shown serious side effects associated with pioglitazone in HIV-infected individuals on HAART. [17]

5. CONCLUSIONS

In conclusion, ritonavir modestly decreased the oral clearance of pioglitazone in rats. The mechanism that underlies the interaction between ritonavir and pioglitazone probably involves the inhibition of CYP3A4-catalysed pioglitazone metabolism by ritonavir. Concomitant administration of ritonavir could thus result in increased plasma concentrations of pioglitazone with increased efficacy and/or adverse events. The dose of pioglitazone is normally higher to achieve a beneficial on hyperlipidamia effects on hyperlipidamia, as if it is taken alongwith ritonnavir in diabetes it increases the level of pioglitazone may develop severe hypoglycaemia. So the study warrants to use relatively less dose of pioglitazone than the normal dose required for the beneficial effect on hyperlipidamia induced by ritonavir. However, extensive clinical pharmacokinetic studies are necessary to establish such drug–drug interactions in diabetic and HIV-infected patients.

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