Effects of Resveratrol on pharmacokinetics and pharmacodynamics of Pioglitazone in Diabetic rats

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ABSTRACT

Introduction: Diabetes is a group of metabolic disorders characterized by a chronic hyperglycaemic condition resulting from insufficient action of insulin. CYP inhibiting drugs increase the concentration of the drugs that are substrates for the specific CYP isoforms and thus enhance the pharmacological and toxicological effects of the substrate drugs. Pioglitazone is a CYP3A4 substrate and resveratrol is a strong inhibitor. Hence, there is a chance of influence on the pharmacokinetics and pharmacodynamics of Pioglitazone.

Materials & Methods: The study was performed on male wistar rats, divided into five groups. Diabetes was induced by administration of Streptozotocin 45mg/kg in 0.1M citrate buffer. Diabetic rats were treated with resveratrol before administration of pioglitazone. Blood samples were collected from orbital puncture at various time intervals and analysed for blood glucose and pharmacokinetics of pioglitazone by HPLC.

Results: serum concentration (mcg/ml) of pioglitazone increased in diabetic rats supplemented with resveratrol.

Conclusion: The results of increased pioglitazone levels as a result of its metabolic inhibition under resveratrol exposure suggests an interaction which may be due to decreased metabolism of pioglitazone as a result of CYP3A4 and CYP2C8 inhibition.

Keywords: Type 2 diabetes, Pioglitazone, Resveratrol, Wistar rats, Pharmacokinetics.

INTRODUCTION

Diabetes is a problem with your body that causes blood glucose levels to rise higher than normal.Type 1 diabetes occurs most frequently in children and young adults, although it can occur at any age. There does appear to be a genetic component to Type 1 diabetes, but the cause has yet to be identified. Type 2 diabetes is much more common and accounts for 90-95% of all diabetes. Type2diabetes primarily affects adults, however recently Type 2 has begun developing in children. There is a strong correlation between Type 2 diabetes, physical inactivity and obesity[1].

Therapy of Type 2 diabetes involves modifications of lifestyle and diet, an exercise regimen, and use of oral hypoglycaemia agents. Observations in the 1940s that certain sulfonamide antibiotics, used to treat typhoid fever and pneumonia, caused the side effect of hypoglycaemia led to the development of the sulfonylurea hypoglycaemic agents.

Pioglitazone hydrochloride a prescription drug of the thiazolidinedione class is an oral antidiabetic agent that acts primarily by decreasing insulin resistance[2].Pharmacological studies indicate that thiazolidinedione improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis. Thiazolidinedione improves glycaemic control while reducing circulating insulin levels.

Serum concentrations of total pioglitazone (pioglitazone plus active metabolites) remain elevated 24 hours after once daily dosing. Steady-state serum concentrations of both pioglitazone and total pioglitazone are achieved within 7 days. At steady-state, two of the pharmacologically active metabolites of pioglitazone, Metabolites III (M-III) and IV (M-IV), reach serum concentrations equal to or greater than pioglitazone. In both healthy volunteers and in patients with type 2 diabetes, pioglitazone comprises approximately 30% to 50% of the peak total pioglitazone serum concentrations and 20% to 25% of the total area under the serum concentration-time curve (AUC). Maximum serum concentration (Cmax), AUC, and trough serum concentrations (Cmin) for both pioglitazone and total pioglitazone increase proportionally at doses of 15mg and 30mg per day. There is a slightly less than proportional increase for pioglitazone and total pioglitazone at a dose of 60mg per day.

Renal elimination of pioglitazone is negligible, and the drug is excreted primarily as metabolites and their conjugates.The mean serum half-life of pioglitazone and total pioglitazone ranges from 3-7 hours and 16-24 hours, respectively.Compared with normal controls, subjects with

impaired hepatic function (Child-Pugh Grade B/C) have an approximate 45% reduction in pioglitazone and total pioglitazone mean peak concentrations but no change in the mean AUC values.

Resveratrol, also known as 3,5,4'-trihydroxystilbene, is a stilbene found in grape skin. It is best known for being found in high concentrations in red wine, but is also present in very small amounts in many other plant products[3].It has received considerable attention by researchers for its anti-inflammatory, anti-tumerogenic, and antioxidant properties[4], as well as its ability to increase lifespan[5].

Resveratrol has received special attention because it has been found to be the most potent activator of SIRT1, a gene critical to aging.Resveratrol decreases nuclear factor kappa B (NFκB) activation.Cancer and other chronic diseases, such as diabetes are associated with chronic activation of NFκB. This chronic activation causes muscle wasting and insulin resistance[1] as well as resistance to cancer chemotherapy[6].

Drug interactions can be pharmacokinetic or pharmacodynamic. Pharmacokinetic interactions result from alterations in a drug's absorption, distribution, metabolism, or excretion characteristics.Pharmacodynamic interactions are a result of the influence of combined treatment at a site of biological activity and yield altered pharmacological actions at standard plasma concentrations.Although drug interactions occur through a variety of mechanisms, the effects are the same: the potentiation or antagonism of the effects of drugs[7].

MATERIALS AND METHODS

Male wistar rats, weighing between 180-250g, were used in the study. They were maintained under standard laboratory conditions at ambient temperature. They were fed with standard pellet diet and water ad libitum. The food was withdrawn from the animal cages 12 hours before experiment and during experiment.

The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethical Committee.

Induction of diabetes in rats

Wistar rats fasted overnight (180-220gms) and diabetes was induced by administration of Streptozotocin 45mg/kg in 0.1M citrate buffer and was administered I.P. Rats were immediately administered with 5% dextrose to antagonise the rapid hypoglycemia effects. Rats were checked for the blood glucose level after 3 days and rats which had blood glucose level >200mg/dl are included in the study.

Pharmacodynamic and Pharmacokinetic interaction study in diabetic rats:

• Group 1: Normal control.

• Group 2: Diabetic control (45mg/kg STZ given in 0.1M sodium citrate buffer).

• Group 3: Pioglitazone (30mg/kg) after induction of diabetes.

• Group 4: Single dose treatment (SDT) studies of resveratrol (50mg/kg) followed by pioglitazone (30mg/kg) in diabetes induced rats.

• Group 5: Multi dose treatment (MDT) Studies resveratrol given for 7 days (50mg/kg) followed by pioglitazone (30mg/kg) in diabetes induced rats.

Blood samples were collected from orbital puncture at time intervals between 0, 0.5,1, 2, 4, 8, 10 and 12 hours using heparinised capillaries. Serum was separated by centrifugation. And blood glucose levels were determined using Glucometer method and remaining serum was stored in vials at -20°C until further analysis.

There are several methods for the estimation of blood glucose. In present study we have used the electrochemical sensoring using a blood glucose monitoring Glucometer

Principle:

The principle behind blood glucose meters is based on reactions that are analyzed by electrochemical sensors. On each strip, there are about 10 layers, including a stiff plastic base plate, and other layers containing chemicals or acting as spacers. There is a layer containing two electrodes (silver or other similar metal).There also is a layer of the immobilized enzyme, glucose oxidase, and another layer containing microcrystalline potassium ferricyanide.Specifically, the reaction of interest is between glucose and glucose oxidase. The glucose in the blood sample reacts with the glucose oxidase to form gluconic acid, which then reacts with ferricyanide to form ferrocyanide. The electrode oxidizes the ferrocyanide, and this generates a current directly proportional to the glucose concentration.

Specimen:

The blood is collected through tail tip method. The tip of the rat tail is pierced and a single drop of blood (approx ˜ 3µl) is collected onto the test trip and the blood glucose levels were read. The Blood Glucose level were also measured through the blood collected by the retro orbital puncturing and taking a single drop of blood on the test tube using a capillary.

Pharmacokinetic evaluation:

HPLC description:

A Shimadzu Class VP series HPLC system with two LC-

10AT pumps, a SPD-10A variable wavelength programmable UV/VIS detector, a CBML-20A prominence communication bus module and a RP C-18 column (Merck,Hiber ; 250 mm×4.6 mm; particle size 5 µm) was used. The system was equipped with LC solutions software.

Preparation of Standard solutions: (ICH GUIDELINES)

Primary stock solutions of pioglitazone and rosiglitazone (Internal standard) were prepared in methanol at a concentration of 1mg/ml and stored at -20^0 C.

Standard Graph procedure

1. Primary stock solution of rosiglitazone was diluted with methanol to obtain the working solution of 100µg/ml concentration. To 100µL of plasma samples, 20µl of internal standard from 100µg/ml of working solution was added to obtain 20µg/ml final concentration and 20µl of pioglitazone.

2. Was added from each concentration to obtain final concentrations 0.1, 0.5, 1, 5, 10 and 50µg/ml of pioglitazone.

3. The resultant solution was mixed for 2 min on cyclomixer at room temperature, and 400µl of methanol was added and centrifuged at 4000 rpm for 10 min the supernatant was separated which is called Supernatant I and 400 µl of methanol was added to residue and the resultant solution was mixed again for 2 min on cyclomixer at room temperature and centrifuged at 4000 rpm for 20 min and then Supernatant was added to the Supernatant I

4. The supernatant I and II are pooled was collected and kept for evaporated to dryness on water bath, the residue was dissolved in 200µl of methanol and after filtration through 0.2 µm syringe filter, 20µl of the solution was spiked for the HPLC analysis.

5. The peak area of the drug and internal standard was determined and the peak area ratio was calculated using the formula

Peak Area Ratio = Peak Area of standard Drug / Peak Area of Internal Standard

6. Graph was plotted by taking concentration on X-axis and peak area ratio on Y-axis.

7. The standard graph was considered to be significant when the r2 value is = 0.99.

Extraction procedures (LLE):

To 100 µL of plasma samples, 20 µL of internal standard from 100 µg/ml of working solution was added and 400µl of methanol was added, the resultant solution was mixed for 2 minutes on cyclomixer at room temperature, and centrifuged at 4000 rpm for 10 min and the Supernatant was separated, which is called Supernatant I and 400µl of methanol was added to residue and the resultant solution was mixed again for 2 min on cyclomixer at room temperature and centrifuged at 4000 rpm for 10 min and then Supernatant was added to the Supernatant I. Now the total volume of the Supernatant is evaporated to dryness on water bath, the residue was dissolved in 200 µL of mobile phase and after filtration through 0.2 µm syringe filter, 20 µl of the solution was used for the HPLC analysis.

Calculations of Pharmacokinetic parameters:

Pharmacokinetic parameters were calculated using "KINETICA" software. All the data were expressed as Mean ± Standard deviation.

RESULTS

The results obtained shows that resveratrol when combined with pioglitazone significantly improves mean serum concentration of pioglitazone. The results are displayed in the following tables and figures

Pharmacokinetic study:

Standard graph of Pioglitazone in rat serum: The equation of the calibration curve obtained was y=mx+c. And it's calibration curve was shown below.

Table.1: Pioglitazone concentrations and PAR in rat serum.

Pioglitazone (PIO) Conc (ug/ml)	Internal standard (IS) conc(ug/ml	Area of pio	Area of IS	Peak Area Ratio (PAR)
0.1	10	3664.1	138271.3	0.0265
0.5	10	10589.8	136211.3	0.0777
1	10	22521.9	129959.3	0.1736
5	10	65361.1	135468.5	0.4826
10	10	170765.2	136258.6	1.2534
50	10	901602.8	129998.4	6.9358

Fig.1: Calibration curve of Pioglitazone in rat serum.

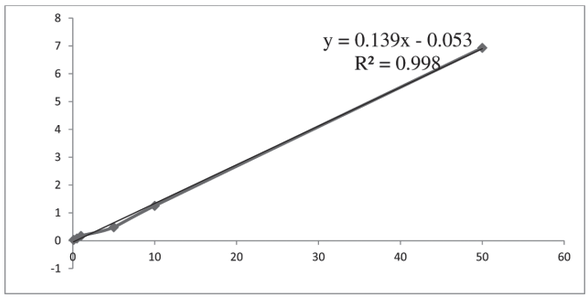


Fig.2: HPLC chromatogram of rat blank serum.

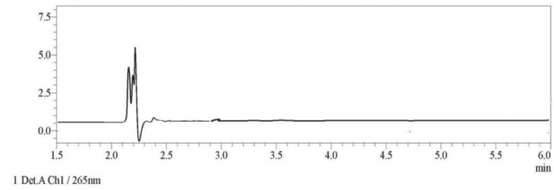
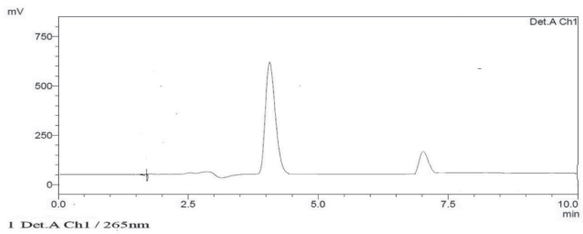


Fig.3: HPLC chromatogram of pioglitazone and rosiglitazone in rat serum.



Mobile phase: Acetonitrinle : 0.5 mM KH2PO4(60:40 v/v)

Flow rate : 1.2 ml/min

λ max : 265 nm

Retention time for pioglitazone: 3.8 min

Retention time for rosiglitazone: 6.8 min

Table.2: Mean serum concentration (ug/ml) of Pioglitazone and pioglitazone in presence of resveratrol (SDT & MDT) in diabetic rats:

TIME (h)	PIO	PIO+RSV (SDI)	PIO+RSV (MDI)
0	0±0	0±0	0±0
1	2.1±0.24	2.8±0.29	3.1±0.28
2	4.6±0.32	6.1±0.35	6.8±0.41
4	6.6± 0.72	8.5± 0.68	9.7±0.9
6	4.3±0.28	6.4±0.31	7.8±0.34
8	3.2±0.18	4.8±0.20	6.1±0.26
10	2.1±0.9	3.5±0.12	4.9±0.23
12	1.3±0.8	2.3±0.2	3.2±0.1

Table.3: Mean Pharmacokinetic parameters of Pioglitazone and Pioglitazone in presence of resveratrol (SDT &MDT) in diabetic rats:

PK PARAMETER	PIO	PIO+RSV (SDT)	PIO+RSV (MDT)
Cmax (µg/ml)	6.6±0.72	8.5±0.7**	9.7±0.9***
tmax(h)	4±0	4±0	4±0
AUC 0-t (µg/ml/h)	48.11±1.8	74.61±2.7***	97.05±3.2***
AUMC 0-t (µg/ml/h*h)	324.95±4.3	602.26±5.7**	894.58±6.09***
t ½(h)	3.07±0.05	4.3±0.06**	5.16±0.09***
MRT (h)	6.75±0.64	8.07±0.71*	9.21±0.83**
Clearance (l/hr)	207.83±5.45	134.01±2.85***	103.03±2.03***
Vd(ml)	922.92±8.68	832.96±7.32***	768.11±6.13***
Vss(ml)	1403±15.64	1081±12.36**	949±7.64**

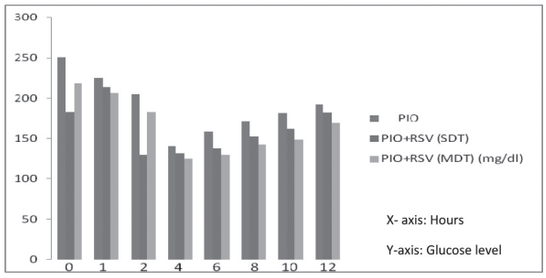
Mean ± SD: ***significant at p<0.001; ** significant at p<0.01; *significant at p<0.05 compared to Pioglitazone control; SDT (Single dose treatment); MDT (Multiple dose treatment). Statistical analysis was performed using One way ANOVA (Dunnetst test).

Pharmacodynamic data:

Table.4: Mean blood glucose levels (mg/dl) in diabetic rats after oral administration of Pioglitazone and pioglitazone in presence of resveratrol (SDT &MDT):

TIME (h)	PIO	PIO+RSV (SDT)	PIO+RSV (MDT)
0	250.67±72	182.67±7.7**	218.0±3.0**
1	224.67±4.7	213.33±4.1*	206.5±00**
2	204.67±8.4	129.67±2.0*	182.67±7.7*
4	140.67±3.0	132.00±2.6*	125.00±4.0**
6	158.67±3.0	138.00±4.0**	129.67±2.0**
8	171.00±4.5	152.67±2.5**	142.33±3.0**
10	181.67±6.6	161.67±3.7**	148.67±5.5**
12	192.43±4.5	182.12±2.1**	169.01±3.1**

Fig. 4: Mean blood glucose levels (mg/dl) in diabetic rats after oral administration of Pioglitazone and pioglitazone in presence of resveratrol (SDT &MDT):



DISCUSSION

Diabetes is a chronic metabolic disorder and needs prolonged treatment for maintenance of normal blood glucose levels. Diabetes may precipitate cardiovascular, renal, neurological disorders.

Pioglitazone is a known hypoglycemic of PPAR-? inhibitors class. It is primarily metabolized by CYP 3A4 and CYP 2C8.

Earlier Neeratietal.,(2014 & 2012) studied inhibition CYP 3A4 by Piperin and Curcummin thereby increasing bioavailability of Pioglitazone. And Suresh etal.,(2009) studied inhibition CYP 3A4 byItraconazole thereby increasing bioavailability of Pioglitazone.

Thus, the present study was done to investigate the influence of resveratrol on the pharmacokinetics and pharmacodynamics of Pioglitazone in-vivo, in streptozotocininduced diabetic rats[8]. In single dose interaction studies, C_{max}, AUC_{tot}, $AUMC_{tot}$, $t_{1/2}$, is increased when compared to diabetic Pioglitazone group. In multi-dose interaction studies,Cmax, AUC_{tot}, $AUMC_{tot}$, $t_{1/2}$, and MRT is significantly increased and clearance significantly decreased when compared to diabetic Pioglitazone group.These results indicate that resveratrol has inhibited the metabolism of Pioglitazone significantly in both single dose and multi dose administration. These changes in the pharmcokinetics and pharmacodynamics of pioglitazone when co-administered with resveratrol may be due to the inhibition of CYP3A4& CYP2C8 enzymes by resveratrol[9].

CONCLUSION

The results of increased pioglitazone levels as a result of its metabolic inhibition under resveratrol exposure suggests an interaction which may be due to decreased metabolism of pioglitazone as a result of CYP3A4 and CYP2C8 inhibition.

Since, the alterations are more pronounced in multiple dose treatment groups, it indicates the significance of long term exposure of resveratrol in diabetic condition being controlled by pioglitazone in rats, and thus it may apply to diabetic patients under pioglitazone treatment.

Hence, the combination has a beneficial effect in diabetic condition, but special concern has to be observed in diabetic patients with cardiovascular complications in view of the side effects of pioglitazone. Hence the present investigation warrants further studies to find out the relevance of this interaction in human beings and postulates the exact mechanism involved.

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