Influence of Piperine on Pioglitazone Metabolism and Pk/Pd: Diabetes Mellitus

Prasad Neerati and Sudhakar A Yakkanti

1Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, India
2Cell signaling Laboratory, Bioscience Division, Center for Cancer and Metabolism, SRI International, USA

Abstract

Background: Piperine is an alkaloidal compound and is an active constituent of black and long peppers. Piperine is known to inhibit cytochrome P-450 isoforms. The objective was to determine the effects of dietary piperine on the pharmacokinetics and pharmacodynamics of pioglitazone.

Methods and Results: Set one non diabetic study, there are three groups, as 1, 2 and 3 composed each of four Albino rabbits (2-3 kg). In the next set, another similar three groups named group 4 group 5 and group 6 were selected to investigate the influences in diabetic group after receiving alloxan monohydrate (80 mg/kg, i.v). Group 1 and group 2; group 4 and group 5 received pioglitazone (10 mg/kg; po) and piperine (20 mg/kg; po) respectively. Group 3 and group 6, we investigated herb drug interactions for single dose and multiple dose interactions on normal and diabetic rabbits with piperine for single day and for eight days. On the last day of piperine pre-treatment, pioglitazone was given. 1 ml blood samples collected intravenously via marginal ear vein, at the pre set time points and PK & PD parameters were measured. Serum levels of pioglitazone measured using RP-HPLC and glucose levels measured. Significant changes observed under multiple dose pre-treatment of piperine. AUCₐ₀ of pioglitazone significantly increased in normal and diabetic rabbits. The biological half-life (t₁/₂) was increased whereas clearance was decreased. Maximum percentage glucose reduction was increased significantly in both single and multiple dose pre-treatment of piperine in diabetic rabbits. The observations suggest an interaction due to metabolic inhibition of CYP enzymes.

Conclusion: Piperine increases the bioavailability of pioglitazone in normal and diabetic rabbits. Piperine, pioglitazone combination has a beneficial effect in diabetes but it may require special care and should be given under medical supervision with appropriate dose combinations in severe diabetes.

Keywords: Piperine; Diabetes Mellitus; Alloxan; Pl/Pd; Pioglitazone; HPLC

Introduction

In some studies piperine is known to inhibit human CYP2C8, CYP3A4 and P-glycoprotein and the enzymes more important for the metabolism of pioglitazone and transport of xenobiotics [1-3]. Piperine can improve the bioavailability of many drugs and decrease the elimination of the drugs and finally improves the biological effectiveness [2]. Major population from America taking herbal medications without informing their medical doctors [4]. Piperine has been found to have anti diabetic activity per se [5]. However, as the metabolism of pioglitazone is inhibited by piperine there is the chance to improve the therapy with this combination.

Pioglitazone is metabolised by CYP3A4 [6] and its metabolism is inhibited by piperine. So there is the chance to potential interactions between piperine and pioglitazone combined usage. Pioglitazone can cause several complications during the treatment of the diabetes mellitus and the combination can reduce the dose and also the complications.

Investigators have predicted that India will have the greatest increase in diabetes and will have the largest number of diabetic patient in the world [7]. It has been reported that Asian Indians have an ethnic susceptibility to type 2 diabetes. The literature indicates that the prevalence of the metabolic syndrome and particularly diabetes is high among migrant Asian Indian and is rising very rapidly even within the Indian sub-continent. Recent WHO reports show that, India already has the largest number of the diabetic patient compared to any given company.

Materials and Method

Animals and diet

Albino white rabbits of male weighing 2-3 kg were used in the study. They were maintained under standard laboratory condition at ambient temperature. They were fed with pellet diet and water ad libitum. The animals were fasted for overnight before experiment and during the experiment they were withdrawn from food and water. The in vivo experimental protocol was approved (IAEC/11/UCPSc/ KU/2011) by the Institutional Animal Ethical committee Kakatiya University, Warangal.

Drugs and chemicals

Pioglitazone and rosiglitazone (internal standard) were the kind gift samples from Dr. Reddy’s Lab (Hyderabad, India). Piperine supplied by Sinthite Pharma, (Kerala, India). Alloxan monohydrate from Sigma-Aldrich (Bangalore, India). Glucose estimation kits were supplied by Excel diagnostics pvt.ltd (Hyderabad, India). Orthophosphoric acid
analytical grade and HPLC grade acetonitrile, methanol and potassium dihydrogen phosphate supplied by Merck (Mumbai, India).

**HPLC analysis of pioglitazone**

Pioglitazone was estimated by an earlier reported modified reverse phase HPLC method [8,9]. HPLC system consisted of LC-10 ATVP solvent delivery module (Shimadzu, Kyoto, Japan), SPD-20AVP variable wavelength programmable UV/VIS spectrophotometric detector, a Class CR-10 Data processor and Reverse Phase C18 column (Wakosil II C-18, 250x4.6 mm, 5 µ porous silica spheres) was used. Rheodyne injection port with a 20 µl sample loop and Hamilton syringe 20 µL was used.

**Chromatographic conditions**

Pioglitazone concentration was determined by slight modification of a method reported the mobile phase consists of 25 mM Phosphate buffer (PH adjusted to 3 with orthophosphoric acid), acetonitrile & methanol in a ratio of 55:37.5:7.5 (v/v/v). The mobile phase was degassed & filtered through 0.22 µm membrane filter. The flow rate was 1.2 mL/min & the effluent was monitored at 269 nm. The total run time of the method was set at 10 min.

**Preparation of standard graph**

The stock solution of pioglitazone & rosiglitazone were prepared in methanol at a concentration of 1 mg/mL each. Rosiglitazone was used as internal standard (IS). By appropriately diluting the stock solutions, different concentrations of pioglitazone (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 µg/mL) & rosiglitazone (10 µg/mL) were prepared.

To a volume of 100 µL of blank rabbit serum, 50 µL of rosiglitazone (2.5 µg in methanol) solution as internal standard & 100 µL of acetonitrile were added to precipitate the proteins. The mixture was vortex mixed for 5 min after which it was centrifuged at 10,000 xg for 10 min. 20 L of the supernatant was injected on to the HPLC system for analysis (Figure 1).

**Limit of detection and Limit of quantification**

Three calibration curves were obtained by spiking thrice, the standard dilutions of pioglitazone in serum samples.

The Equation of I calibration curve of pioglitazone: \[ y=0.179x - 0.0782 \]

The Equation of II calibration curve of pioglitazone: \[ y=0.179x - 0.0793 \]

The Equation of III calibration curve of pioglitazone: \[ y=0.1778x - 0.0843 \]

\[ \sigma=0.003251 \]

\[ S=0.1786 \]

\[ \sigma \rightarrow \text{Standard deviation of } y \text{- intercepts of Calibration Curves.} \]

\[ m \rightarrow \text{Mean of the slopes of Calibration Curves of Pioglitazone.} \]

\[ \text{L.O.D}=3.3 \sigma / S \]

\[ =0.060072 \]

The L.O.D of Pioglitazone from the Equations of the three Calibration curves of Pioglitazone was found to be 0.060072 µg.

\[ \text{L.O.Q}=10 \sigma / S \]

\[ =0.18204 \]

\[ \sigma \rightarrow \text{Standard deviation of } y \text{- intercepts of Calibration Curves.} \]

\[ m \rightarrow \text{Mean of the slopes of Calibration Curves of Pioglitazone.} \]

The L.O.Q of Pioglitazone from the Equations of the three Calibration curves of Pioglitazone was found to be 0.18204 µg.

Hence, the limit of detection and limit of quantification were both found to be within the range of the analytic levels in serum samples.

**Accuracy and precision**

Intra and inter-day precision expressed as percentage of standard deviation.
Assay precision was calculated using the formula:
\[
\% \text{RSD} = \left( \frac{SD}{M} \right) \times 100
\]
where SD is the standard deviation of M.

Accuracy was calculated using the formula:
\[
\% \text{RE} = \left( \frac{E - T}{T} \right) \times 100
\]
where E is the experimentally determined concentration and T is the theoretical concentration.

Intra and inter day precision and accuracy of the determination of pioglitazone quality control samples (n = 4). The assay procedure was found to be precise and accurate.

### Calculations of pharmacokinetic parameters

Non compartmental Pharmacokinetic analysis was carried out using Kinetica TM software (version 4.4.1 Thermo Electron Corporation, U.S.A). The following Pharmacokinetic parameters were calculated: C<sub>max</sub>, T<sub>max</sub>, t<sub>1/2</sub>, AUC<sub>0-inf</sub>, AUC<sub>inf</sub>, t<sub>MRT</sub>, Cl, Vd and Vdss. Mean glucose levels and percentage reduction in blood glucose concentrations were determined for the pharmacodynamic data.

% glucose reduction at t hour = \[(G_t - G_0) / G_t\] × 100

\(G_t\) → mean glucose levels at t hour

\(G_0\) → mean glucose levels at 0 hour

### Statistical analysis

The results expressed as mean ± SD. The difference in between concentration time profiles; in between pharmacokinetic parameters; in between serum glucose levels and difference between over the entire range tested were analyzed by one-way ANOVA (Bonferroni post-test) (rockville1985). The differences were considered to be significant at P<0.05.

### Results

The serum concentration levels of pioglitazone in normal rabbits were read out by substituting the peak area ratio values of each sample in the equation obtained from the calibration curve of pioglitazone

\[\text{Serum concentration} = \frac{\text{Peak area ratio}}{\text{Standard curve slope}}\]

\(\text{Peak area ratio}\) of the sample

\(\text{Standard curve slope}\) in the calibration equation

\(\text{Serum concentration}\) in μg/mL

The following Pharmacokinetic parameters were calculated:

\[\{C_{\text{max}} , T_{\text{max}} , AUC_{\text{0-inf}} , AUC_{\text{inf}}, AUMC_{\text{0-inf}}, AUMC_{\text{inf}}, t_{1/2}, \text{MRT}, \text{Cl}, \text{Vd and Vdss}\}

The results obtained from the pharmacokinetic analysis were found to be consistent with the pharmacodynamic findings.
pioglitazone. The mean values along with standard deviation were calculated for each time point (Table 1) and a concentration versus time curve was obtained by plotting the mean concentration of pioglitazone at 2 hr in mean serum concentration levels of pioglitazone at 2 hr (from 0.81 ± 0.34 to 1.36 ± 0.19; p<0.05 Table 3) and a significant increase in AUC_{0 to ∞} (from 12.65 ± 2.97 to 19.70 ± 3.16; p<0.05 Table 3). Significant changes in pharmacokinetic parameters. The Tmax of pioglitazone extended from 0.5 hr to 1 hr under piperine pretreatment.

Pharmacokinetics of pioglitazone in non diabetic and diabetic rabbits with piperine

The pharmacokinetic parameters for pioglitazone were calculated and showed a C_{max} of 2.03 ± 0.34 mg/ml, T_{max} of 0.5 hrs and AUC of 9.21 ± 1.12 mg/hr ml (Table 2) in normal rabbits.

Pharmacokinetics of pioglitazone under piperine pretreatment in normal rabbits

The mean serum concentration levels of pioglitazone in normal rabbits under piperine pretreatment were also read out (Table 3), a comparative concentration versus time curves of all groups was obtained (Figure 3) and the pharmacokinetic parameters of all groups were also compared (Table 4). In normal rabbits, pretreatment of piperine resulted in an increase in mean serum concentration of pioglitazone at 1 hr in single dose interaction group (from 1.03 ± 0.32 to 2.04 ± 0.34; p<0.05); at 1 & 2 hr in multiple dose interaction group (from 1.03 ± 0.32 to 2.06 ± 0.44 & from 0.70 ± 0.21 to 1.18 ± 0.45 respectively; p<0.05 Table 2) and significant changes in pharmacokinetic parameters. The T_{max} of pioglitazone extended from 0.5 hr to 1 hr under piperine pretreatment.

Pharmacokinetics of pioglitazone in non diabetic and diabetic rabbits with piperine

The pharmacokinetic parameters for pioglitazone were calculated and showed a C_{max} of 2.01 ± 0.87 mg/ml, T_{max} of 0.5 hrs and AUC of 12.65 ± 1.97 mg/hr ml in normal rabbits. The T_{max} of pioglitazone extended from 0.5 hr to 1 hr under piperine pretreatment. In diabetic rabbits, multiple dose pretreatment of piperine resulted in an increase in mean serum concentration levels of pioglitazone at 2 hr (from 0.81 ± 0.34 to 1.36 ± 0.19; p<0.05 Table 3) and a significant increase in AUC_{0 to ∞} (from 12.65 ± 2.97 to 19.70 ± 3.16; p<0.05 Table 3). Significant changes in pharmacokinetic parameters. The Tmax of pioglitazone extended from 0.5 hr to 1 hr under piperine pretreatment.

Table 1: Mean serum concentration of pioglitazone in non diabetic and diabetic rabbits.

<table>
<thead>
<tr>
<th>Time(hr)</th>
<th>Non Diabetic</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>0.5</td>
<td>2.03049 ± 0.34364</td>
<td>2.0157 ± 0.31452</td>
</tr>
<tr>
<td>1</td>
<td>1.03281 ± 0.32064</td>
<td>1.15479 ± 0.21450</td>
</tr>
<tr>
<td>2</td>
<td>0.70300 ± 0.21542</td>
<td>0.81124 ± 0.34001</td>
</tr>
<tr>
<td>4</td>
<td>0.60084 ± 0.22886</td>
<td>0.75146 ± 0.30092</td>
</tr>
<tr>
<td>8</td>
<td>0.43687 ± 0.22446</td>
<td>0.64006 ± 0.25617</td>
</tr>
<tr>
<td>24</td>
<td>0.10687 ± 0.04611</td>
<td>0.20100 ± 0.05145</td>
</tr>
</tbody>
</table>

Mean ± SD

Table 2: Mean serum concentration of pioglitazone in presence of piperine (SDI & MDI) in non diabetic and diabetic rabbits.

<table>
<thead>
<tr>
<th>Time(hr)</th>
<th>Non Diabetic</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>0.5</td>
<td>2.03049 ± 0.34364</td>
<td>2.0157 ± 0.31452</td>
</tr>
<tr>
<td>1</td>
<td>1.03281 ± 0.32064</td>
<td>1.15479 ± 0.21450</td>
</tr>
<tr>
<td>2</td>
<td>0.70300 ± 0.21542</td>
<td>0.81124 ± 0.34001</td>
</tr>
<tr>
<td>4</td>
<td>0.60084 ± 0.22886</td>
<td>0.75146 ± 0.30092</td>
</tr>
<tr>
<td>8</td>
<td>0.43687 ± 0.22446</td>
<td>0.64006 ± 0.25617</td>
</tr>
<tr>
<td>24</td>
<td>0.10687 ± 0.04611</td>
<td>0.20100 ± 0.05145</td>
</tr>
</tbody>
</table>

Mean ± SD; ***significant at p<0.001; **significant at p<0.01; *significant at p<0.05 compared to pioglitazone control; SDI: Single Dose Interaction; MDI: Multiple dose interaction; PIO: pioglitazone; PIP: Piperine.
The mean glucose levels and percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated. The mean glucose levels and percentage glucose reduction was compared in piperine pretreatment against control group in normal rabbits. Pretreatment of piperine resulted in an increase in percentage glucose reduction more in 8 & 24 hr (from 11.83 to 25.48 & 1.74 to 8.76 respectively; p<0.05 Table 4) in multiple dose group of normal rabbits. The mean glucose levels and percentage glucose reduction was compared in piperine pretreatment against control group in diabetic rabbits. The mean glucose levels and percentage glucose reduction was calculated in pharmacokinetic parameters including decrease in clearance and increase in t1/2 under multiple dose exposure. There was an increase in mean serum levels of pioglitazone in both normal and diabetic rabbits and also on the pharmacokinetic parameters under piperine pretreatment.

**Pharmacodynamic data**

Effects of piperine on the pharmacodynamics of pioglitazone in non diabetic and diabetic rabbits: The mean blood glucose levels for each time point in normal rabbits were calculated using glucose oxidase peroxidase method and the percentage glucose reduction at each time point compared to the mean glucose levels at 0 hr was calculated. The mean glucose levels and percentage glucose reduction were observed in pharmacokinetic parameters including decrease in clearance and increase in t1/2 under multiple dose exposure. There was an increase in mean serum levels of pioglitazone in both normal and diabetic rabbits and also on the pharmacokinetic parameters under piperine pretreatment.

**Discussion**

Diabetes is a chronic metabolic disorder and needs prolonged
Piperine resulted in an increase in AUCtot by 0.59 folds (p<0.05), multiple doses pretreatment. In normal rabbits, pre-treatment of piperine on the pharmacokinetics and pharmacodynamics of pioglitazone in rabbits. The pharmacokinetic parameters were found to alter in normal rabbits, multiple dose pretreatment of piperine resulted in an increase in AUCtot by 0.82 folds (p<0.01), AUMC tot by 1.56 folds (p<0.01), t1/2 by 0.34 folds (p<0.05), MRT by 0.31 folds (p<0.05) and a decrease in Cl by 0.36 folds (p<0.05). In diabetic rabbits also maximum percentage glucose observed at 4 hr in all groups of normal rabbits and any changes were less significant. In diabetic rabbits also maximum percentage glucose reduction was observed at 4 hr and the influence of piperine was found to be statistically significant in both single dose pretreatment (at 4, 8, 24 hr by 0.38, 0.23, 1.52 folds respectively; p<0.05) and multiple dose pretreatment (at 2, 4, 8, 24 hr by 0.73, 0.67, 0.50, 3.14 folds respectively; p<0.01). The increase in AUC only in multiple dose treatment suggests an inhibitory influence of piperine on hepatic metabolism. Also, influence of piperine being effective in improving pharmacodynamics (glycemic control) only in diabetic rabbits indicates that the alteration might be partly because of improved pharmacokinetics of pioglitazone and partly because of antihyperglycemic activity of piperine. Thus, the improved pharmacokinetic parameters of pioglitazone was more observed in the multiple dose treatment groups, and the improvement of pharmacodynamics was significant in only diabetic rabbits under multiple dose treatment, thus showing the significance of influence of piperine in multiple dose exposure.

Conclusions

The results of increased AUC of pioglitazone under piperine exposure suggest an interaction which may be due to decreased metabolism of pioglitazone as a result of CYP 3A4 and 2C8 inhibition. Since the alterations are more pronounced in multiple dose treatment groups, it indicates the significance of long term exposure of piperine in diabetic condition being controlled by pioglitazone in rabbits, and thus it may apply to diabetic patients under pioglitazone treatment. Hence, the combination has a beneficial effect in diabetic condition, but therapeutic drug monitoring has to be observed in view of 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.

Acknowledgement

This research work was funded by All India Council for Technical Education (AICTE), New Delhi, India. The authors are thankful to AICTE (8023/BOR/RID/RPS-107/2009-10).

References

