Altered Pharmacokinetics and Pharmacodynamics of Glimepiride by the concomitant use of Quercetin in diabetic rats: PK/PD modeling

Sujatha Samala and Ciddi Veeresham*
University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana State - 506009, India

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ABSTRACT

The effect of quercetin on the pharmacokinetics (PK) and pharmacodynamics (PD) of glimepiride was studied in normal and streptozotocin induced diabetic rats. In normal and diabetic rats the combination of glimepiride with quercetin increased significantly (p < 0.01) all the pharmacokinetic parameters, such as \( C_{\text{max}} \), AUC\(_{0-n}\), AUC\(_{\text{total}}\), \( t_{\frac{1}{2}} \), MRT and decreased the clearance, \( V_d \) markedly as compared with the control. In pharmacodynamic studies, the increase in hypoglycemic action by concomitant administration of glimepiride with quercetin was more in diabetic rats than when the drugs were used singly and with the control group, which suggests the enhancement of glucose reduction capacity of glimepiride in diabetic rats along with quercetin. In addition, the combination of glimepiride with quercetin also improved the total antioxidant status in diabetic rats compared with quercetin and glimepiride alone treated groups. In PK/PD modeling of quercetin with glimepiride, the predicted PK and PD parameters are in line with the observed PK and PD parameters. The results revealed that quercetin led to the PK/PD changes have been due to glimepiride increased bioavailability, decreased total clearance and volume of distribution may be due to the inhibition of cytochrome P450 metabolic system. Hence, glimepiride doses may require special attention if administered concomitantly with quercetin or quercetin containing herbal preparations to avoid complications. This could be important in reducing the dose of glimepiride to achieve desired therapeutic effect with minimal adverse effects.

KEYWORDS: Quercetin, glimepiride, pharmacokinetics, pharmacodynamics, cytochrome P450, PK/PD modeling

1. INTRODUCTION

Diabetic patients often consume herbal preparations along with routinely prescribed antidiabetic agents.\(^1\) Such herbal preparations often contain bioflavonoids, which not only confer per se antidiabetic effect but also eliminate hyperglycemia - induced oxidative stress, and thereby help to attenuate secondary complications of diabetes. Glimepiride, a sulphonylurea used as an oral hypoglycemic agent, is widely used for the treatment of type 2 diabetes mellitus. The hypoglycemic effect of glimepiride was changed during co-administration with Carica papaya extract,\(^2\) thus there is a need to study the interaction between glimepiride and other drugs to avoid adverse effects.

Quercetin is a dietary flavonoid, which is present in many antidiabetic herbal preparations. It is widely distributed mainly as glycosides in components of the daily diet such as onions, apples, berries, black tea, red wine and in various fruit juices. Quercetin is reported to have various biological activities such as anti-inflammatory,\(^3\) antioxidant,\(^4\) antitumourigenic\(^5\) and antidiabetic.\(^6\) There are several in-vitro reports of quercetin on inhibition of CYP450s especially CYP3A4,\(^7\) CYP2C8\(^8\) and CYP2C9.\(^9\) Hence there is the possibility of quercetin for the metabolic inhibition of glimepiride, which is also completely metabolized by CYP2C9 microsomal liver enzymes.\(^10\)

In view of the influence of quercetin on CYP enzymes especially on CYP2C9 and also its bioenhancer, antidiabetic properties, its presence in herbal antidiabetic preparations may alter the pharmacokinetics and pharmacodynamics of glimepiride, particularly because the later is metabolized by CYP2C9. Therefore, the aim of the present investigation was to study the effect of quercetin on pharmacokinetics and pharmacodynamics of glimepiride and also PK/PD modeling of these interactions for quantitative representation of the dose - concentration - response relationship, which provide information for prediction of the level of response to a certain level of drug dose.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals

Glimepiride and gliclazide were obtained as gift samples from Dr. Reddy’s laboratories (Hyderabad, India). Quercetin, \( \alpha, \alpha’\) - diphenyl -
2.2. Maintenance of animals
Male Albino rats of Wistar strain weighing 180-250g were purchased from Mahaveera enterprises, Hyderabad, India and used for the studies after obtaining the permission from institutional animal ethical committee (CPCSEA Reg. No.146/1999). The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12:12 h light and dark cycle; at an ambient temperature of 25 ± 5°C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water ad libitum.

2.3. Pharmacokinetic study

2.3.1. Grouping of normal rats and pretreatment
Rats were divided into 2 groups (n = 6). Group I was administered with glimepiride (1 mg/kg; p.o.) suspended in normal saline on 8th day. Group II was pretreated with quercetin (20 mg/kg; p.o.) for 7 days and on 8th day with glimepiride (1 mg/kg) followed by quercetin.

Blood samples were collected from retro - orbital vein puncture using heparinised capillary tubes at 0.5, 1, 2, 4, 6, 8, 12 and 24 h. Serum was separated after centrifugation at 8000 rpm for 15 min and stored at -20°C until analysis.

2.3.2. Induction of diabetes in rats
Diabetes was induced by using streptozotocin (55 mg/kg, b.w., i.p.) in citrate buffer (pH 4.5) to the overnight fasted Wistar rats. After 72 h, blood samples were collected from rats by retro orbital puncture and the serum was analyzed for glucose levels. Animals with blood glucose level > 250 mg/dl were considered as diabetic and were used for the study.

2.3.3. Grouping of diabetic rats and treatment
Diabetic rats were divided into 2 groups (n = 6) and were treated; blood samples were collected as mentioned for normal rats.

2.3.4. HPLC analysis of glimepiride in normal and diabetic pre-treated rats
Serum glimepiride concentration was determined by reverse phase HPLC. The HPLC system was equilibrated with the mobile phase consisting of methanol: 10mm potassium dihydrogen ortho phosphate (pH 3.0 adjusted with ortho phosphoric acid) (80:20 v/v), at a flow rate of 1.0 ml/min.

2.4. Pharmacodynamic studies

2.4.1. Effect of quercetin with glimepiride on serum glucose in diabetic rats
STZ - induced diabetic rats were fasted overnight and divided into 4 groups (n = 6). The animals of group I (diabetic control, normal saline), group II (glimepiride, 1 mg/kg), group III (quercetin, 20 mg/kg) and group IV [quercetin (20 mg/kg) + glimepiride (1 mg/kg)] were treated orally. Blood samples were drawn from the retro - orbital plexus of the rats at ‘0’ (Initial fasting blood sample), 2, 4, 6, 8, 12 and 24 h after the treatment. The samples were analyzed for blood glucose using glucose oxidase - peroxidase method.

2.4.2. Estimation of total antioxidant status in diabetic pretreated rats
Overnight fasted diabetic rats were divided into 4 groups (I-IV) same as mentioned in the antihyperglycemic study and treated once a day for 28 days (sub acute study) and the effect of quercetin, glimepiride and their combination on total antioxidant status was observed up to 28 days. The serum samples of sub acute study were used to determine the total antioxidant status by using DPPH method. Ascorbic acid was used as a reference standard. The standard graph was prepared using different concentrations of ascorbic acid in water (y = 0.0018 x + 0.0116, r = 0.9953) and the antioxidant status values were expressed in terms of nM of ascorbic acid.

2.4.3. Estimation of lipid peroxide levels in diabetic pretreated rats
The serum samples of sub acute study were used to determine the lipid peroxides by using Thiobarbituric acid reaction method. The standard graph for determination of malondialdehyde levels was prepared using 1,1,3,3 - tetraethoxy propane (TEP) reagent as the standard (y = 0.075 x - 0.0368, r = 0.9989) and the MDA content in the serum was expressed in nM/ml.

2.5. Pharmacokinetic and pharmacodynamic modeling
The pharmacokinetic and pharmacodynamic modeling of glimepiride in control and quercetin treated STZ - induced diabetic rats was performed by fitting plasma concentration - time data of glimepiride to a one compartment open model following oral administration by using WinNonlin software (version 5.2, Pharsight, USA). The pharmacodynamic model used to fit the serum glucose concentrations was a direct effect model linked to open one compartment PK model with an equilibration rate constant (Ke0) i.e. in this link model the first order equilibration rate constant was used to link pharmacokinetic compartment with the effect (pharmacodynamic) compartment.
2.6. Statistical analysis
The Pharmacokinetic parameters were calculated by using Kinetta TM software (version 4.4.1, Thermo Electron Corporation, USA). All values of pharmacokinetic and pharmacodynamic studies were expressed as mean ± SD. The data were statistically evaluated using one way analysis of variance (ANOVA) followed by post hoc Dunnet’s t-multiple comparison test using Graph Pad Prism 5 computer software. Values corresponding to p ≤ 0.05 were considered as significant.

3. RESULTS

3.1. Pharmacokinetics of glimepiride pretreated with quercetin in normal and diabetic rats
Table 1 summarizes the pharmacokinetic parameters of glimepiride in different groups of normal and diabetic rats. The co-administration of Table 1 summarizes the pharmacokinetic parameters of glimepiride in normal and diabetic rats.

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Glimepiride Normal</th>
<th>Glimepiride Diabetic</th>
<th>Glimepiride +Quercetin Normal</th>
<th>Glimepiride +Quercetin Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax(µg/ml)</td>
<td>7.4±0.1</td>
<td>11.3±0.3</td>
<td>10.7±0.5**</td>
<td>22.9±1.8**</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AUC_{(µg/ml h)}</td>
<td>24.7±0.6</td>
<td>51.1±2.1</td>
<td>50.1±1.6**</td>
<td>95.3±5.7**</td>
</tr>
<tr>
<td>AUC_{(µg/ml h)}</td>
<td>24.6±0.7</td>
<td>51.8±2.0</td>
<td>52.4±1.7**</td>
<td>98.9±5.7**</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>1.6±0.2</td>
<td>1.7±0.2</td>
<td>2.26±0.1*</td>
<td>2.7±0.03**</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.8±0.4</td>
<td>3.95±0.1</td>
<td>4.93±0.06**</td>
<td>5.6±0.1**</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>38.7±2.4</td>
<td>18.6±2.4</td>
<td>18.5±12**</td>
<td>7.9±4.2**</td>
</tr>
<tr>
<td>Vd (ml)</td>
<td>90.4±7.5</td>
<td>44.8±7.5</td>
<td>60.3±1.35**</td>
<td>29.7±3.7**</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD (n = 6), *p < 0.05; **p < 0.01 considered as significant when compared with glimepiride control. Definitions of the parameters: Cmax: Peak serum concentration; Tmax: Time to reach peak serum concentration; AUC_{(µg/ml h)}: Area under serum concentration/time plot until the last quantifiable value; AUC_{(µg/ml h)}: Area under serum concentration/ time plot extrapolated to infinity; t_{1/2}: Terminal half life; MRT: Average mean residence time; CL: Total clearance; Vd: Volume of distribution.

3.2. Effect of quercetin and their combination on the hypoglycemic action of glimepiride
The mean serum glucose level and percentage glucose reduction of antihyperglycemic study of pretreated diabetic rats is shown in Table 2. The data reveals that there is a maximum reduction of serum glucose level in quercetin - glimepiride pretreated group (56.16%), when compared to standard (glimepiride, 50.07%) and quercetin (43.52%) alone pretreated groups at 6th h. However, combination of quercetin with glimepiride showed sharp decrease (p < 0.01) in serum glucose levels at all the time points.

Table 2. Comparison of mean serum glucose levels and percentage reduction of serum glucose level of different groups in STZ - induced diabetic rats.

<table>
<thead>
<tr>
<th>Group Treatment No</th>
<th>Dose mg/kg</th>
<th>Blood glucose level (mg/dl) at different hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>I Control</td>
<td>-</td>
<td>334.52±8.64</td>
</tr>
<tr>
<td>II Glimepiride</td>
<td>1</td>
<td>342.53±5.4</td>
</tr>
<tr>
<td>III Quercetin</td>
<td>20</td>
<td>340.68±7.47</td>
</tr>
<tr>
<td>IV Quercetin+</td>
<td>20+1</td>
<td>303.18±8.59</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD (n = 6), *p < 0.05; **p < 0.01 considered as significant when compared with Group I at respective time interval.

3.3. Total antioxidant status and lipid peroxide levels of different groups in diabetic rats
The serum total antioxidant status and lipid peroxide levels of different pretreated glimepiride groups in diabetic rats is shown in Fig. 1 and Fig. 2. The effect of the quercetin - glimepiride group found gradually increased (p < 0.01) in total antioxidant status when compared with quercetin, glimepiride alone pretreated groups and with control group at all time intervals of the study. The lipid peroxide levels were found decreased significantly (p < 0.01) in quercetin - glimepiride group when compared with quercetin, glimepiride alone pretreated groups and with control group at all time intervals of the study.

Fig. 1. Effect of different groups on total antioxidant status in sub acute study.
3.4. PK/PD modeling of quercetin - glimepiride interactions

Mean observed and predicted serum glucose levels at different time intervals of glimepiride alone and with quercetin are shown in Fig. 3 and Fig. 4. Different pharmacokinetic and pharmacodynamic model parameters of glimepiride in control (glimepiride alone treated) and quercetin treated diabetic rats are presented in Table 3 and the predicted pharmacodynamic effect of serum glucose levels is shown in Fig. 5. In glimepiride alone treated rats the volume of distribution (Vd) was found to be 53.32 ml/kg, whereas in quercetin treated rats the glimepiride pharmacokinetic parameter Vd was observed to be 30.59 ml/kg, respectively.

**Table 3. Pharmacokinetic and pharmacodynamic modeling of different parameters of glimepiride in different groups of diabetic rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>PK parameters</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLM</td>
<td>Vd (ml/kg)</td>
<td>53.32</td>
<td>0.8</td>
<td>8.27</td>
</tr>
<tr>
<td></td>
<td>$K_{a1}$ (1/h)</td>
<td>0.76</td>
<td>0.24</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>$K_{e1}$ (1/h)</td>
<td>0.42</td>
<td>0.1</td>
<td>1.16</td>
</tr>
<tr>
<td>GLM+QR</td>
<td>Vd (ml/kg)</td>
<td>30.59</td>
<td>7.16</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td>$K_{a1}$ (1/h)</td>
<td>0.39</td>
<td>0.03</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>$K_{e1}$ (1/h)</td>
<td>0.29</td>
<td>0.05</td>
<td>1.48</td>
</tr>
</tbody>
</table>

**Definitions of the parameters:**

- $V_d$: Volume of distribution
- $K_{a1}$: Absorption rate constant
- $K_{e1}$: Elimination rate constant
- $E_{0}$: Baseline effect (serum glucose levels)
- $IC_{50}$: Half maximal inhibitory concentration
- $K_{e0}$: First order equilibration rate constant
- $Imax$: Maximum fractional extent of inhibition

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**Fig. 2. Effect of different groups on lipid peroxide levels in sub acute study.**

**Fig. 3. Mean observed and predicted serum glucose levels vs time in glimepiride alone pretreated group. Points represent the observations. The dotted line represents a cubic spline through the observations. The solid line represents a cubic spline through the predictions.**

**Fig. 4. Mean observed and predicted serum glucose levels vs time in glimepiride along with quercetin pretreated group. Points represent the observations. The dotted line represents a cubic spline through the observations. The solid line represents a cubic spline through the predictions.**

**Definitions of the parameters:**

- $E_0$: Base line effect (serum glucose levels)
- $IC_{50}$: Half maximal inhibitory concentration
- $K_{e0}$: First order equilibration rate constant
- $Imax$: Maximum fractional extent of inhibition.
In normal and STZ-induced diabetic rats, combination of glimepiride with quercetin led to a significant increase in pharmacokinetic parameters such as $C_{\text{max}}$, $AUC_{0-24h}$, $AUC_{\text{total}}$, $t_{1/2}$ and MRT. This may be due to alteration in the metabolism of glimepiride either by enhancing absorption or by inhibiting CYP2C9 responsible for glimepiride metabolism. There is no change in $T_{\text{max}}$ of glimepiride in both normal and diabetic rats indicating that there is no alteration in rate of absorption of glimepiride and the serum affinity of glimepiride for albumin is 99.5% bound. Acidic drugs can displace ionic binding of sulfonylureas from serum proteins to far greater extent than the non-ionic bound glimepiride. This indicates that the decreased volume of distribution may not be due to displacement of glimepiride by quercetin. As there is no plasma protein binding interactions between quercetin and glimepiride, the decreased volume of distribution may be due to metabolic inhibition of glimepiride by quercetin. The results are in agreement with earlier reports, that quercetin increases the bioavailability of tamoxifen, pioglitazone, diltiazem, and also in accordance with the earlier in-vitro studies of quercetin metabolic inhibition on CYP2C9 enzyme in human liver microsomes.

The increase in hypoglycemic action by concomitant administration of glimepiride with quercetin was more in diabetic rats than when the drugs were used singly and with the control group, which suggests the enhancement of glucose reduction capacity of glimepiride in diabetic rats along with quercetin. In case of determination of oxidative stress markers quercetin offer significant protection against the oxidative stress induced by diabetes in combination with glimepiride and interferes with PK and PD.

In PK/PD modeling, the volume of distribution in the combination of glimepiride with quercetin treated rats was significantly ($p < 0.01$) decreased, when compared with the glimepiride alone treated rats. Whereas the baseline response (blood glucose levels) was decreased in combination of quercetin with glimepiride group as well as in glimepiride alone treated group, because quercetin and glimepiride both exhibits antidiabetic activity. Hence, in PK/PD modeling of quercetin - glimepiride interactions the predicted PK and PD parameters are in line with the observed PK and PD parameters.

5. CONCLUSION
It was concluded that careful addition of quercetin in moderate doses might result in beneficial effect during treatment of diabetes in patients, when compared to glimepiride alone treatment in rats. This could be important in reducing the dose of glimepiride to achieve desired therapeutic effect with minimal adverse effects.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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