

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/biochempharm

Quercetin pretreatment increases the bioavailability of pioglitazone in rats: Involvement of CYP3A inhibition

Sudhir N. Umathe*, Pankaj V. Dixit, Vijendra kumar, Kuldeep U. Bansod, Manish M. Wanjari

Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj (RTM) Nagpur University, Mahatma Jyotiba Fuley Shaikshanik Parisar, Amravati Road, Nagpur 440 033, India

ARTICLE INFO

Article history:

Received 4 January 2008

Accepted 22 January 2008

Keywords:

Cytochrome P450 3A (CYP3A)

Herb–drug interactions

Diabetes mellitus

Pharmacokinetics

HPLC

ABSTRACT

Herbal antidiabetic preparations are often used as an add-on therapy in diabetes and such herbal preparations often contains quercetin that can inhibit cytochrome P450 (CYP) 3A4. This enzyme is responsible for metabolizing pioglitazone, a commonly used antidiabetic agent. Hence, it was speculated that quercetin may influence the bioavailability of pioglitazone, which could be particularly crucial, as any increment in its plasma levels may raise safety concerns. Thus, we first established the inhibitory influence of quercetin (2, 10 and 20 mg/kg, p.o.) on CYP3A activity by an in vivo method of estimating levels of midazolam in female rats pretreated with dexamethasone. It was further confirmed in vitro using erythromycin-N-demethylase (EMD) assay. These studies indicated potent inhibition of CYP3A activity by quercetin (10 and 20 mg/kg, in vivo; 1 and 10 μ M, in vitro). In another experiment, pioglitazone was administered orally (10 mg/kg) and intravenously (5 mg/kg) to quercetin (10 mg/kg) pretreated female rats and its plasma levels were determined at various time points (0.5, 1, 2, 4, 8 and 24 h after oral administration; 0.083, 0.5, 1, 2, 8, 12 and 18 h after i.v. administration) by HPLC. Quercetin pretreatment increased $AUC_{0-\infty}$ of pioglitazone after oral administration by 75% and $AUC_{0-\infty}$ after intravenous administration by 25% suggesting decreased metabolism, which could be due to inhibition of CYP3A by quercetin. In conclusion, add-on preparations containing quercetin may increase the bioavailability of pioglitazone, and hence should be cautiously used.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

Pioglitazone, a thiazolidinedione derivative decreases insulin resistance via its action at the peroxisome proliferator-activated receptor subtype gamma (PPAR- γ), and emerged as a novel oral antidiabetic agent in recent past [1,2]. 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 [3,4]. A recent report suggests that rifampicin; an inducer of CYP3A4 decreased the area under the curve (AUC) of pioglitazone by 35% whereas, gemfibrozil; an inhibitor of CYP2C8 increased the AUC by 239% [5]. 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.

It is often evident that diabetic patients often consume herbal preparations along with routinely prescribed antidiabetic agents [6–8]. Such herbal preparations often contain

* Corresponding author. Tel.: +91 712 2500324; fax: +91 712 2500355.

E-mail address: umathesn@hotmail.com (S.N. Umathe).

0006-2952/\$ – see front matter © 2008 Elsevier Inc. All rights reserved.

doi:10.1016/j.bcp.2008.01.010

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 [9]. Some flavonoids such as chrysin, apigenin and kampferol are even reported to be potent PPAR- γ agonists [10].

Quercetin is one such naturally occurring dietary flavonoid, ubiquitously present in many antidiabetic herbal preparations, and even in black tea, red wine and various fruit juices [11]. Incidentally, quercetin is reported to inhibit the activity of CYP3A4 in vitro with IC_{50} of 38 μ M [12,13]. On the contrary, one scanty report indicates CYP3A4 induction in human hepatocytes [14]. Probably therefore, quercetin was found to increase the plasma concentration of CYP3A4 substrates such as acyclovir [15]; saquinavir [16]; ritonavir [17]; tamoxifen [18] and paclitaxel [19]. In addition, quercetin is also reported to inhibit P-glycoprotein (Pgp), an efflux protein in intestine, and thus demonstrated to increase the bioavailability of Pgp substrates such as moxidectin [20]; digoxin [21] and diltiazem [22].

In view of the influence of quercetin on CYP3A activity, its presence in herbal antidiabetic preparations may influence the pharmacokinetics of pioglitazone, particularly because the later is metabolized by CYP3A. Therefore, the aim of the present investigation was to study the influence of quercetin on pharmacokinetics of pioglitazone.

The studies were first directed to find the dose of quercetin that would inhibit CYP3A activity, which was studied by an in vivo method, wherein plasma levels of orally administered midazolam (a CYP3A substrate) are measured in dexamethasone pretreated rats [23]. As in vivo animal studies alone cannot be predictive of the influence of quercetin on CYP3A activity in humans, it was further confirmed by an in vitro method of erythromycin-N-demethylase (EMD) assay [24]. Later, rats were treated with inhibitory dose of quercetin, and its influence on the pharmacokinetics of orally and intravenously 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 Sprague–Dawley rats as an animal model [25].

2. Materials and methods

2.1. Materials

Quercetin monohydrate was purchased from Sigma–Aldrich (St. Louis, MO, USA). Dexamethasone and midazolam were gifted by Wochardt Research Center, Aurangabad, India. All other chemicals used were of analytical grade. Freshly double distilled deionised water, filtered through 0.45 μ m Nylon filter (47 mm) (Pall Life Sciences, Mumbai, India) in Millipore unit (USA), was used throughout the experiments.

The drug analysis was carried out using HPLC system (Shimadzu 10AT/Vp, Kyoto, Japan) having in-built degasser unit (DGU 14A), mixer unit (FCV 10AL) attached to solvent delivery module with low-pressure gradient pump (LC 10AT), Rheodyne injector port (2E, 7725i, 20 μ l loop), column oven (CTO 10AS) and UV/vis detector (SPD 10A Vp). The data interpretation was done with CSW (Chromatographic work station) (Shimadzu, Kyoto, Japan) data acquisition software.

2.2. Animals

Female Sprague–Dawley rats (250–300 g) were purchased from National Centre for Laboratory Animal Sciences, Hyderabad, India, and acclimatized to our animal house for at least 10 days prior to the experiments. Throughout the experiment, three animals were housed per cage, and maintained at $25 \pm 2^\circ$ C, 50–60% relative humidity. They were fed standard rodent diet (Trimurti Feeds, Nagpur, India) with water ad libitum. The Institutional Animal Ethics Committee approved the use of animals for the present investigations.

2.3. Study design

2.3.1. Assessment of in vivo CYP3A activity

In vivo CYP3A activity was assessed by using a model developed by Kanazu et al. [23]. The method is based on the principle that midazolam being a substrate for CYP3A, the activity of this enzyme inversely reflects upon the plasma levels of orally administered midazolam. CYP3A activity is artificially induced by dexamethasone pretreatment so that the inhibitory effect of any agent would better reflect on midazolam levels. The present studies employed female Sprague–Dawley rats as CYP3A isoform is predominant in this gender.

In brief, female Sprague–Dawley rats were divided in six groups ($n = 3$). Five groups received dexamethasone (80 mg/kg, i.p.) daily for three consecutive days while one group received vehicle and served as dexamethasone untreated control group. After 24 h of last dose of dexamethasone, quercetin (2, 10 and 20 mg/kg) or reference standard (ketoconazole, 5 mg/kg) was orally administered. Control group received vehicle (0.5% methylcellulose in water). It is reported that orally administered quercetin is completely absorbed in 1 h [11]. Therefore, after 1 h of quercetin administration, midazolam (20 mg/kg, p.o.) was administered. Blood samples (~ 0.3 ml) were collected in heparinized eppendorf tubes at 15, 30 min, 1, 1.5, 2 and 4 h through tail vein and centrifuged immediately at $3000 \times g$ for 15 min to separate plasma which was stored at -20° C until analysis.

The plasma concentration of orally administered midazolam was determined by HPLC [26,27]. In brief, plasma (0.1 ml), internal standard (0.1 ml of diazepam; 80 ng in methanol) and NaOH (0.5 ml, 1N) were mixed together. Then *n*-hexane (3.0 ml) was added to the mixture, vortex mixed for 5 min and later centrifuged at $10,000 \times g$ for 5 min. The upper layer (3.0 ml) was separated and evaporated under vacuum. The residue was dissolved in 100 μ l of mobile phase and 20 μ l was injected onto the HPLC system for analysis. The UV-detector was set at 230 nm. SYMMETRY[®] C₈ column (4.6 mm \times 150 mm, 5 μ m, Waters Co., Milford, USA) was used at a temperature of 30° C. Mobile phase consisted of 0.1 mM sodium acetate buffer (pH 4.7) and acetonitrile in a ratio of 60:40 (v/v). The flow rate was maintained at 1.0 ml/min. The method was linear in the range of 50–10,000 ng/ml.

2.3.2. In vitro assessment of CYP3A activity in liver and intestine

The effect quercetin on CYP3A activity in intestinal and liver microsomes was studied by an in vitro method developed by Wrighton et al. [24]. The method is based on the principle that

CYP3A4 converts erythromycin to N-demethyl erythromycin and formaldehyde, which produces yellow color with Nash reagent [28].

After overnight fasting, rats were euthanized by pento-barbitone overdose. Liver was perfused with 10 ml of 0.1 M phosphate buffered saline (PBS) and then isolated. Similarly, a piece of intestine (~25 cm) was isolated and washed with PBS. Both the tissues were stored at -20°C until further processing.

2.3.2.1. Preparation of intestinal microsomes. A method validated by Cotreau et al. and Takemoto et al. was employed with slight modifications to prepare intestinal microsomes [29,30]. In brief, the isolated intestine was cut into pieces, washed with ice cold PBS and then cut longitudinally to expose mucosa. The mucosal layer was scraped lightly from all pieces of intestine with the help of a cover slip. All scrapings were mixed together and centrifuged at $25 \times g$ for 5 min. The pellet was suspended in 5.0 ml of ice cold histidine–sucrose buffer (HSB) (histidine 5 mM, pH 7.0; sucrose 0.25 M; NaEDTA 0.5 mM; pH 7.4); homogenized and centrifuged at $15,000 \times g$ for 10 min. The supernatant was carefully transferred to a clean tube. The pellet was resuspended in 5.0 ml of HSB and again centrifuged at $15,000 \times g$ for 10 min. The supernatant after each centrifugation was taken together and mixed with 52 mM CaCl_2 (0.2 ml per ml of the supernatant) to precipitate microsomes. After 15 min of standing, it was centrifuged at $20,000 \times g$ for 15 min, the microsomal pellet was suspended in 0.5 ml of 0.1 M potassium phosphate buffer containing 20% glycerol, and stored at -20°C until needed. Protein concentration of the microsomal fraction was determined by Biuret method using bovine serum albumin as the standard.

2.3.2.2. Preparation of liver microsomes. The liver microsomes were prepared by slight modification of the method proposed by Schenkman and Cinti [31]. The liver isolated from rats was minced and homogenized in appropriate amount of 0.25 M sucrose containing 10 mM Tris–HCl (pH 7.4), and then centrifuged at $600 \times g$ for 5 min followed by $12,000 \times g$ for 10 min. The post-mitochondrial supernatant was separated, mixed with solid CaCl_2 so that its concentration in the given volume of supernatant was 8.0 mM and then centrifuged at $20,000 \times g$ for 20 min. The pellet so obtained was resuspended in mixture of 150 mM KCl–10 mM Tris–HCl, and centrifuged at $20,000 \times g$ for 20 min to obtain pinkish microsomal pellet, which was suspended in 0.5 ml of 0.1 M potassium phosphate buffer containing 20% glycerol and stored at -20°C until needed. Protein concentration of samples was determined by Biuret method using bovine serum albumin as the standard.

2.3.2.3. Erythromycin-N-demethylation assay. The mixture of microsomal suspension (0.1 ml, 25%), erythromycin (0.1 ml, 10 mM) and potassium phosphate (0.6 ml, 100 mM, pH 7.4) was incubated at 37°C along with quercetin at a concentration of 0.1, 1 and 10 μM . The reaction between these agents was initiated by adding NADPH (0.1 ml, 10 mM), and terminated after 10 min, by adding ice cold trichloroacetic acid (0.5 ml, 12.5%, w/v) solution. It was centrifuged ($2000 \times g$; 10 min) to remove proteins. To 1.0 ml of this supernatant 1.0 ml of Nash Reagent (2 M ammonium acetate, 0.05 M glacial acetic acid, 0.02 M acetylacetone) was added, and heated in a water bath at

50°C for 30 min. After cooling, the absorbance was read at 412 nm. The activity was calculated from standards (1–100 μM formaldehyde) prepared by substituting sample with standard solution which were run in parallel. The CYP3A4 activity was expressed as nM of formaldehyde obtained per milligram of protein per hour.

2.3.3. Pharmacokinetics of pioglitazone in quercetin treated rats

After overnight fasting, two groups each of female SD rats ($n = 4$) were treated with quercetin (10 mg/kg as suspension in 0.5% methylcellulose) or vehicle. The dose selection of quercetin for the present study was based upon the observations from the earlier experiments. After 1 h of quercetin or vehicle administration, pioglitazone (10 mg/kg orally in 0.1 M citrate buffer) was orally administered to both groups. In remaining two groups, pioglitazone (5 mg/kg) was intravenously administered through saphenous vein.

Blood (~0.3 ml) was withdrawn through tail vein, and collected in heparinized tubes at 0.5, 1, 2, 4, 8 and 24 h after oral administration, and 0.083, 0.5, 1, 2, 8, 12, and 18 h after intravenous administration of pioglitazone. Blood samples were immediately centrifuged at $3000 \times g$ for 15 min to obtain plasma, and stored at -20°C until analysis.

Pioglitazone concentration was determined by slight modification of a method reported by Kolte et al. [32]. In brief, to 100 μl of plasma sample, 50 μl of rosiglitazone (12.5 μg in methanol) solution as internal standard and 100 μl of acetonitrile were added to precipitate the proteins. The mixture was vortex mixed for 5 min after which it was centrifuged at $10,000 \times g$ for 10 min. 20 μl of the supernatant was injected onto the HPLC system for analysis. The UV detector was set at 269 nm for the present analysis. C18(2) column (4.6 mm \times 250 mm, 100 Å) Luna, PHENOMENEX[®], USA was set at 30°C . The flow rate was 1.2 ml/min and the mobile phase consisted of 25 mM phosphate buffer (pH 3.0), acetonitrile and methanol in a ratio of 70:25:5 (v/v/v). The method was linear over 0.625–20 $\mu\text{g/ml}$.

2.4. Data analysis

2.4.1. Pharmacokinetic analysis

Plasma concentration–time curve was plotted, and the maximum plasma concentration (C_{max}), time needed to reach the maximum plasma concentration (T_{max}) for midazolam and pioglitazone was noted; area under the concentration–time curve ($\text{AUC}_{0-\infty}$) and mean residence time (MRT) was calculated using non-compartmental pharmacokinetic model of WINNONLIN[®] (Version 1.5, SCI Software, Statistical Consulting, Inc., Apex, NC, USA). The elimination rate constant (K_{el}) was calculated by the software using slope of the terminal elimination phase; and half-life was calculated by $0.693/K_{\text{el}}$. Absolute bioavailability was calculated as follows.

$$\text{Absolute bioavailability (AB\%)} \\ = (\text{AUC}_{\text{oral}}/\text{AUC}_{\text{iv}}) \times (\text{IV dose/oral dose}) \times 100$$

2.4.2. Statistical analysis

All the means are presented with their standard deviation (mean \pm S.D.). The pharmacokinetic parameters of midazolam

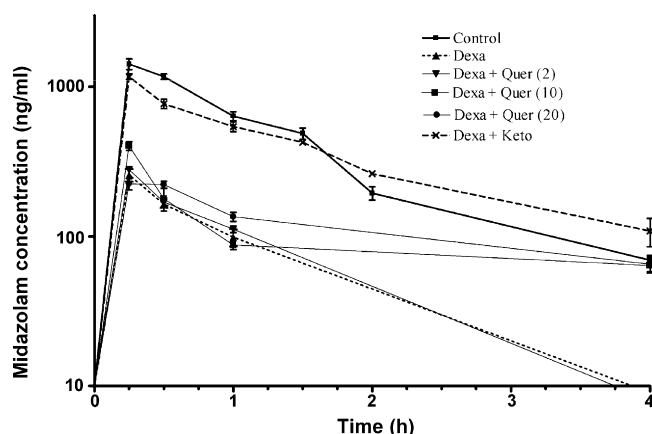


Fig. 1 – Plasma concentration–time curve of midazolam after its oral administration (20 mg/kg) to dexamethasone/vehicle pretreated rats. Each point represents the mean \pm standard deviation ($n = 3$).

in vehicle and quercetin/ketoconazole treated groups were compared using one-way ANOVA, followed by post hoc Dunnett test. Erythromycin-N-demethylase activity in vehicle and quercetin treated groups were compared using same statistical tests. An unpaired Student's *t*-test was used to determine the significant difference between the pharmacokinetic parameters of pioglitazone in control and quercetin treated groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Assessment of in vivo CYP3A activity

Fig. 1 represents the plasma concentration–time profile of midazolam in non-dexamethasone treated control group, and dexamethasone along with quercetin (2, 10 and 20 mg/kg), ketoconazole (5 mg/kg) and vehicle (0.5% methylcellulose) treated group. Table 1 summarizes the pharmacokinetic

parameters of midazolam in all these groups. Dexamethasone pretreatment significantly decreased C_{max} ($P < 0.001$), $AUC_{0-\infty}$ ($P < 0.001$), and MRT ($P < 0.001$) of midazolam as compared to non-dexamethasone treated control group. Administration of quercetin (10 mg/kg) to dexamethasone pretreated rats significantly increased the C_{max} ($P < 0.001$), $AUC_{0-\infty}$ ($P < 0.01$), MRT, K_{el} , $T_{1/2}$ ($P < 0.001$). Higher dose of quercetin (20 mg/kg) significantly increased ($P < 0.001$) all these parameters except C_{max} , and at a lower dose (2 mg/kg) it had no effect ($P > 0.05$). Administration of ketoconazole to dexamethasone pretreated rats similarly significantly increased all pharmacokinetic parameters of midazolam.

3.2. Assessment of in vitro CYP3A activity

Fig. 2 exhibits the extent of erythromycin-N-demethylation (EMD) due to CYP3A activity. One-way ANOVA indicates that quercetin treatment significantly influenced the activity in both intestine ($F(3, 20) = 361.3$, $P < 0.0001$) and liver microsomes ($F(3, 20) = 35.92$, $P < 0.0001$). The post hoc test further reveals that in quercetin treated group the levels of EMD at all concentrations (0.1, 1, and 10 μ M) were significantly reduced ($P < 0.01$) in the intestinal microsomes when compared with vehicle control. Similarly, quercetin treatment decreased EMD in liver microsomes at 1 μ M ($P < 0.05$), and 10 μ M ($P < 0.01$) while it has no significant effect ($P > 0.05$) at 0.1 μ M.

3.3. Pharmacokinetics of pioglitazone in quercetin treated rats

The effect of quercetin administration (10 mg/kg, p.o.) on the pharmacokinetics of oral and intravenous pioglitazone is shown in Figs. 3 and 4, and the pharmacokinetic parameters are depicted in Table 2. Student's *t*-test revealed a significant influence of pretreatment of quercetin on oral pharmacokinetics of pioglitazone. As compared to control group the $AUC_{0-\infty}$ in quercetin pretreated group was significantly increased (d.f. = 6, $P = 0.0019$); the K_{el} (d.f. = 6, $P = 0.0223$), and oral clearance (d.f. = 6, $P = 0.0008$) were significantly decreased; whereas it had no effect on the C_{max} , T_{max} as well as MRT

Table 1 – Pharmacokinetic parameters of midazolam in dexamethasone pretreated female rats

Parameters	Midazolam control	Dexamethasone pretreatment (80 mg/(kg day), i.p. for consecutive 3 days)				
		Vehicle (10 ml/kg)	Quercetin (2 mg/kg)	Quercetin (10 mg/kg)	Quercetin (20 mg/kg)	Ketoconazole (5 mg/kg)
C_{max} (ng/ml)	1170 \pm 85.04	257 \pm 25.24 [®]	279.03 \pm 13.87	402.7 \pm 45.79*	243.3 \pm 11.59	770 \pm 96.99*
K_{el} (1/h)	0.70 \pm 0.04	1.23 \pm 0.31	1.18 \pm 0.22	0.41 \pm 0.07*	0.32 \pm 0.05*	0.71 \pm 0.14 [§]
$AUC_{0-\infty}$ (ng h/ml)	1510 \pm 133.8	232 \pm 27.5 [®]	258.3 \pm 22.37	568.7 \pm 67.1 [§]	667.3 \pm 113.7*	1421 \pm 151.9*
$T_{1/2}$ (h)	0.98 \pm 0.06	0.57 \pm 0.14	0.60 \pm 0.12	1.71 \pm 0.30*	2.17 \pm 0.31*	0.98 \pm 0.18
T_{max} (h)	0.5 \pm 0	0.25 \pm 0	0.25 \pm 0	0.25 \pm 0	0.25 \pm 0	0.5 \pm 0
MRT (h)	1.24 \pm 0.05	0.49 \pm 0.01 [®]	0.49 \pm 0.00	1.53 \pm 0.11*	1.64 \pm 0.06*	1.53 \pm 0.06*
RB (%)	100	15	17	38	44	94

Dexamethasone was administered for three consecutive days and 24 h thereafter vehicle (10 ml/kg) or quercetin (2, 10 and 20 mg/kg) or ketoconazole (5 mg/kg) was orally administered; and 1 h thereafter pharmacokinetics of midazolam (20 mg/kg, p.o.) was studied. T_{max} : time to reach C_{max} ; C_{max} : peak plasma concentration; AUC: area under the plasma concentration curve; K_{el} : elimination rate constant; $T_{1/2}$: half life; MRT: mean residence time; RB: relative bioavailability. All values are mean \pm standard deviation ($n = 3$). [®] $P < 0.001$ when compared to control group; * $P < 0.001$, [§] $P < 0.01$, [®] $P < 0.05$ when compared with dexamethasone pretreated control (vehicle treated) group (one-way ANOVA followed by Dunnett post hoc test).

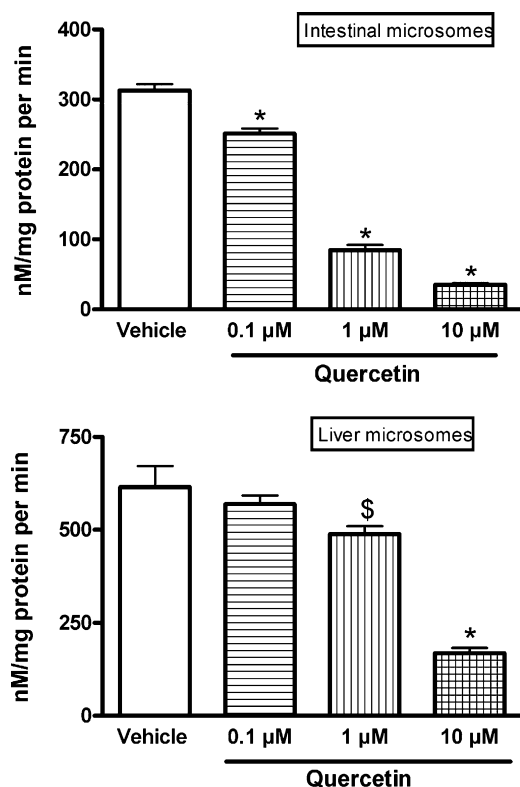


Fig. 2 – Influence of quercetin on CYP3A activity in liver and intestinal microsomes. Each bar represents mean \pm standard deviation of six observations. * $P < 0.01$, \$ $P < 0.05$ vs. respective control group (One-way ANOVA followed by Dunnett test).

($P > 0.05$). Similarly, quercetin pretreatment also had a significant influence on the pharmacokinetics of intravenously administered pioglitazone. The intravenous $AUC_{0-\infty}$ (d.f. = 6, $P = 0.0175$), MRT (d.f. = 6, $P = 0.0111$) and $T_{1/2}$ (d.f. = 6, $P = 0.0391$) in quercetin pretreated group were significantly

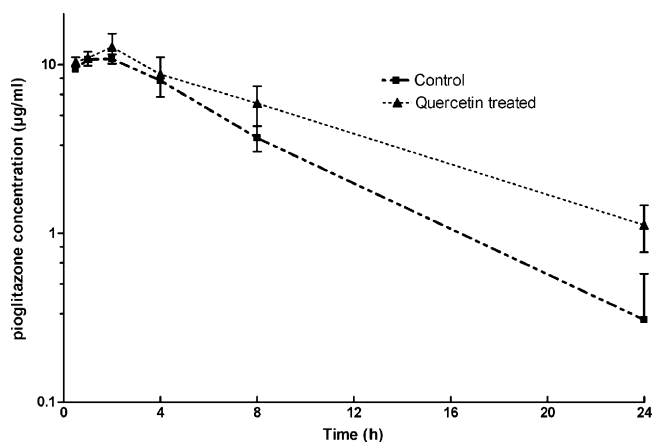


Fig. 3 – Plasma concentration–time curve of pioglitazone after its oral administration (10 mg/kg) to quercetin (10 mg/kg)/vehicle pretreated rats. Each point represents the mean \pm standard deviation ($n = 4$).

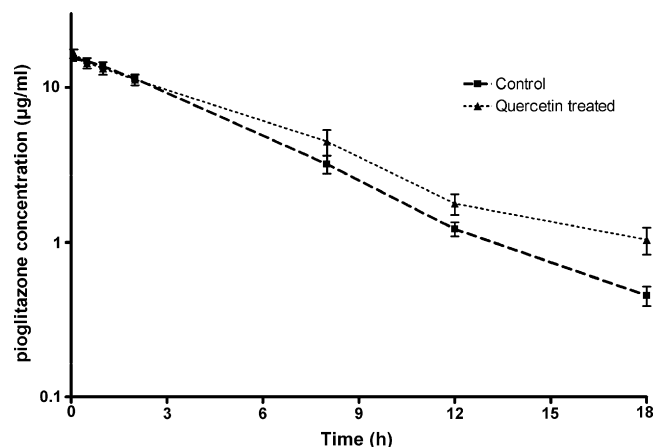


Fig. 4 – Plasma concentration–time curve of pioglitazone after its intravenous administration (5 mg/kg) to quercetin (10 mg/kg)/vehicle pretreated rats. Each point represents the mean \pm standard deviation ($n = 4$).

increased, and the K_{el} (d.f. = 6, $P = 0.025$) and clearance (d.f. = 6, $P = 0.0294$) were significantly reduced.

4. Discussion

The use of complementary therapies for treatment of diabetes is ever increasing, and often remains unnoticed by a physician [8,33]. Furthermore, now-a-days the antidiabetic pharmacological strategy is becoming increasingly complex, and the recommended global approach of combination drug therapy has increased the risk of pharmacokinetic interactions in diabetic patients [34]. Although the risk of hypoglycemia with thiazolidinediones appears negligible but drug interactions may exacerbate adverse effects and raise safety concerns [5]. Several studies have reported that quercetin is a relatively potent inhibitor of CYP3A4 in vitro, which plays a role in the metabolism of pioglitazone. However, slight confusion prevails over whether quercetin has inhibitory actions or stimulatory actions on CYP3A. Accordingly, the present study was designed to clarify the effect of quercetin on the CYP3A activity and, further to test the hypothesis that pioglitazone pharmacokinetics may be altered in the presence of quercetin, as it is metabolized via CYP3A4 in humans.

The present investigations revealed that quercetin dose dependently inhibited the CYP3A enzyme activity, as indicated by increase in the area under the curve ($AUC_{0-\infty}$) of midazolam in dexamethasone pretreated rats. Further, the influence of 10 and 20 mg/kg dose were found to be more or less same. The C_{max} in quercetin treated group at a dose of 20 mg/kg was surprisingly lower than that at 10 mg/kg. However, the $AUC_{0-\infty}$ was about 20% higher at 20 mg/kg. Incidentally, some studies indicate modulation of gastric emptying after quercetin treatment [11]. Although these investigations were not made, but such effect of quercetin may explain the results.

The dexamethasone pretreated female rat model is widely employed for evaluation of CYP3A inhibitors [35]. In this

Table 2 – Pharmacokinetic parameters of pioglitazone in quercetin pretreated rats

Parameters	Pioglitazone IV 5 mg/kg		Pioglitazone PO 10 mg/kg	
	Vehicle treated	Quercetin treated (10 mg/kg, p.o.)	Vehicle treated	Quercetin treated (10 mg/kg, p.o.)
T _{max} (h)	–	–	1.63 ± 0.75	1.75 ± 0.50
C _{max} (μg/ml)	15.67 ± 0.72	16.98 ± 1.09	13.00 ± 1.50	13.29 ± 1.49
AUC _{0–∞} (μg h/ml)	80.95 ± 10.95	98.44 ± 3.86*	77.66 ± 23.49	123.1 ± 26.12*
K _{el} (l/h)	0.19 ± 0.03	0.13 ± 0.02*	0.22 ± 0.08	0.10 ± 0.01*
T _{1/2} (h)	3.61 ± 0.46	5.02 ± 0.96*	3.82 ± 2.47	6.75 ± 0.91
V _z /F (l)	0.33 ± 0.05	0.36 ± 0.08	0.66 ± 0.20	0.78 ± 0.13
Cl/F (l/h)	0.062 ± 0.008	0.050 ± 0.001*	0.14 ± 0.03	0.08 ± 0.02*
MRT (h)	4.87 ± 0.36	6.65 ± 0.91*	6.09 ± 2.95	9.89 ± 1.25
AB (%)	–	–	47.96 ± 14.5	76.03 ± 16.13*

After overnight fast the rats were treated with quercetin (10 mg/kg, p.o.) and 1 h thereafter oral/intravenous pharmacokinetics of pioglitazone was carried out. T_{max}: time to reach C_{max}; C_{max}: peak plasma concentration; AUC: area under the plasma concentration curve; K_{el}: elimination rate constant; T_{1/2}: half life; V_z/F: oral volume of distribution; Cl/F: oral clearance; MRT: mean residence time; AB: absolute bioavailability. All values are mean ± standard deviation (n = 4) *P < 0.05 vs. vehicle treated control group (two-tailed Student t-test).

model, as plasma concentration of midazolam depends upon CYP3A activity, the observed AUC_{0–∞} of midazolam in dexamethasone treated animals indicate that dexamethasone indeed enhanced the CYP3A activity, and the same was inhibited by quercetin and ketoconazole. The observed effects of dexamethasone as well as ketoconazole on the AUC of midazolam are well in accordance with the earlier reports [23].

Further, we investigated the influence of quercetin on erythromycin-N-demethylation assay, which is an indicator of CYP3A activity. The results indicate that the intestinal CYP3A activity was inhibited to greater extent than the liver microsomal activity, which may be attributed to the fact that intestinal microsomal preparations have a low overall concentration of CYPs in comparison to hepatic preparations [29]. These studies further confirm the inhibitory influence of quercetin on CYP3A activity.

The quercetin–pioglitazone pharmacokinetic interaction studies further revealed that administration of quercetin (10 mg/kg) 1 h prior to pioglitazone increased AUC_{0–∞} of orally as well as intravenously administered pioglitazone. The oral AUC_{0–∞} was increased by 75% whereas the intravenous AUC_{0–∞} was increased by 25%. The increase in oral AUC_{0–∞} can be attributed to a decline in oral as well as hepatic clearance. Moreover, the intravenous pharmacokinetic study revealed the extent of inhibition of hepatic metabolism of pioglitazone by quercetin. Due to the increase in the plasma levels of pioglitazone by inhibition of CYP-mediated metabolism the T_{1/2} as well as MRT was increased. However, the C_{max} was unaffected.

Recently, it was estimated that heavy-end consumer of quercetin would be exposed to more than 226 mg of dietary quercetin/person per day [11]. Similarly, it is reported that the dose of quercetin reportedly consumed by diabetic patients is about 500 mg/day. In view of the present observations, the pharmacokinetics of pioglitazone in such individuals is likely to be different. The estimated human equivalent dose of quercetin that may alter the pharmacokinetics of pioglitazone comes out to be 1.6 mg/kg. The present study also revealed that the absolute bioavailability of pioglitazone in control rats is about (~50%), and the clinical evidences indicate comparatively higher bioavailability of pioglitazone in human (~80%)

[3]. However, as the clearance of pioglitazone was altered after both oral and intravenous administration such interaction appears feasible in humans. Finally, quercetin is also reported to have inhibitory influence on CYP2C9 [36] and CYP2C8 [37], which are reported to participate in the hepatic metabolism of pioglitazone. In the absence of any in vitro studies, the contribution of CYP2C8 and CYP2C9 inhibition by quercetin in the present investigations cannot be completely ruled out.

Concluding, quercetin is a potent inhibitor of CYP3A isoform in rats, and increases the bioavailability of concomitantly administered pioglitazone. Hence, pioglitazone doses may require special attention if used along with quercetin containing herbal preparations to avoid the complications due to the increased bioavailability. However, extensive clinical pharmacokinetic studies are necessary to establish such drug–drug interactions in diabetic patients.

Acknowledgement

The authors acknowledge the research grant [vide F31-79/2005(SR)] from University Grants Commission, India.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2008.01.010.

REFERENCES

- [1] Mankovsky B, Kurashvili RB. Glitazones: beyond glucose lowering! *Diab Metab Syndr Clin Res Rev* 2007;1:197–207.
- [2] Elte JW, Blicke JF. Thiazolidinediones for the treatment of type 2 diabetes. *Eur J Intern Med* 2007;18:18–25.
- [3] Gillies PS, Dunn CJ. Pioglitazone *Drugs* 2000;60:333–43.
- [4] Actos product information. Available from: URL: <http://www.actos.com/pi.pdf>. Accessed December 12; 2007.
- [5] Scheen AJ. Pharmacokinetic interactions with thiazolidinediones. *Clin Pharmacokinet* 2007;46:1–12.

- [6] Chavez ML, Jordan MA, Chavez PI. Evidence-based drug-herbal interactions. *Life Sci* 2006;78:2146–57.
- [7] Dunning T. Complementary therapies and diabetes. *Complement Ther Nurs Midwifery* 2003;9:74–80.
- [8] Peng CC, Glassman PA, Trilli LE, Hunter JH, Good CB. Incidence and severity of potential drug-dietary supplement interactions in primary care patients. *Arch Intern Med* 2004;164:630–6.
- [9] Dias AS, Porawski M, Alonso M, Marroni N, Collado PS, Gonzalez-Gallego J. Quercetin decreases oxidative stress, NF- κ B activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. *J Nutr* 2005;135:2299–304.
- [10] Kota BP, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. *Pharmacol Res* 2005;51:85–94.
- [11] Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. *Food Chem Toxicol* 2007;45:2179–205.
- [12] Miniscalco A, Lundahl J, Regårdh CG, Edgar B, Eriksson UG. Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. *J Pharmacol Exp Ther* 1992;261:1195–9.
- [13] Guengerich FP, Kim DH. In vitro inhibition of dihydropyridine oxidation and aflatoxin B1 activation in human liver microsomes by naringenin and other flavonoids. *Carcinogenesis* 1990;11:2275–9.
- [14] Raucy JL. Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products. *Drug Metab Dispos* 2003;31:533–9.
- [15] Yang ZG, Meng H, Zhang X, Li XD, Lv WL, Zhang Q. Effect of quercetin on the acyclovir intestinal absorption. *Beijing Da Xue Xue Bao* 2004;36:309–12.
- [16] Eagling VA, Profit L, Back DJ. Inhibition of the CYP3A4-mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by grapefruit juice components. *Brit J Clin Pharmacol* 1999;48:543–52.
- [17] Patel J, Buddha B, Dey S, Pal D, Mitra AK. In vitro interaction of HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. *Am J Ther* 2004;11:262–77.
- [18] Shin SC, Choi JS, Li X. Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats. *Int J Pharm* 2006;313:144–9.
- [19] Choi JS, Jo BW, Kim YC. Enhanced paclitaxel bioavailability after oral administration of paclitaxel or prodrug to rats pretreated with quercetin. *Eur J Pharm Biopharm* 2004;57:313–8.
- [20] Dupuy J, Larrieu G, Sutra JF, Lespine A, Alvinerie M. Enhancement of moxidectin bioavailability in lamb by a natural flavonoid: quercetin. *Vet Parasitol* 2003;112:337–47.
- [21] Wang YH, Chao PD, Hsiu SL, Wen KC, Hou YC. Lethal quercetin-digoxin interaction in pigs. *Life Sci* 2004;74:1191–7.
- [22] Choi JS, Li X. Enhanced diltiazem bioavailability after oral administration of diltiazem with quercetin to rabbits. *Int J Pharm* 2005;297:1–8.
- [23] Kanazu T, Yamaguchi Y, Okamura N, Baba T, Koike M. Model for the drug-drug interaction responsible for CYP3A enzyme inhibition II: establishment and evaluation of dexamethasone-pretreated female rats. *Xenobiotica* 2004;34:403–13.
- [24] Wrighton SA, Schuetz EG, Watkins PB, Maurel P, Barwick J, Bailey BS, et al. Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol Pharmacol* 1985;28:312–21.
- [25] Fujita Y, Yamada Y, Kusama M, Yamauchi T, Kamon J, Kadowaki T, et al. Sex differences in the pharmacokinetics of pioglitazone in rats. *Comp Biochem Physiol C Toxicol Pharmacol* 2003;136:85–94.
- [26] Yamano K, Yamamoto K, Kotaki H, Takedomi S, Matsuo H, Sawada Y, et al. Quantitative prediction of metabolic inhibition of midazolam by erythromycin, diltiazem, and verapamil in rats: implication of concentrative uptake of inhibitors into liver. *J Pharmacol Exp Ther* 2000;292:1118–26.
- [27] Jurica J, Dostalek M, Konecny J, Glatz Z, Hadasova E, Tomandl J. HPLC determination of midazolam and its three hydroxy metabolites in perfusion medium and plasma from rats. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007;852:571–7.
- [28] Nash T. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* 1953;55:416–21.
- [29] Cotreau MM, von Moltke LL, Beinfeld MC, Greenblatt DJ. Methodologies to study the induction of rat hepatic and intestinal cytochrome P450 3A at the mRNA, protein, and catalytic activity level. *J Pharmacol Toxicol Methods* 2000;43:41–54.
- [30] Takemoto K, Yamazaki H, Tanaka Y, Nakajima M, Yokoi T. Catalytic activities of cytochrome P450 enzymes and UDP-glucuronosyltransferases involved in drug metabolism in rat everted sacs and intestinal microsomes. *Xenobiotica* 2003;33:43–55.
- [31] Schenkman JB, Cinti DL. Preparation of microsomes with calcium. *Methods Enzymol* 1978;52:83–9.
- [32] Kolte BL, Raut BB, Deo AA, Bagool MA, Shinde DB. Liquid chromatographic method for the determination of rosiglitazone in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;788:37–44.
- [33] Dey L, Attele AS, Yuan CS. Alternative therapies for type 2 diabetes. *Altern Med Rev* 2002;7:45–58.
- [34] Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: update regarding thiazolidinediones: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2008;31:173–5.
- [35] Marathe PH, Rodrigues AD. In vivo animal models for investigating potential CYP3A- and Pgp-mediated drug-drug interactions. *Curr Drug Metab* 2006;7:687–704.
- [36] He N, Edeki T. The inhibitory effects of herbal components on CYP2C9 and CYP3A4 catalytic activities in human liver microsomes. *Am J Ther* 2004;11:206–12.
- [37] Walsky RL, Gaman EA, Obach RS. Examination of 209 drugs for inhibition of cytochrome P4502C8. *J Clin Pharmacol* 2005;45:68–78.