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Effect of quercetin on the disposition kinetics of ciprofloxacin

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Abstract

Pharmacokinetic interaction between ciprofloxacin, an antibacterial fluoroquinolone and flavonoid quercetin was investigated in the present study. Male wistar albino rats, weighing about 200-250 g were randomly divided into two groups, six rats in each group. Rats in group I (control) received ciprofloxacin alone (10 mg/kg PO). Group II received ciprofloxacin 30 min after pretreatment with quercetin (20 mg/kg PO). Rats were anaesthetized after ciprofloxacin administration for blood collection with a xylazine-ketamine combination. Blood samples were collected from jugular vein and tail clipping at predetermined time intervals prior to and at 0.33, 0.67,1, 1.5, 2, 4, 6, 8 and 12 h (20, 40, 60, 90, 120, 240, 360, 480 and 720 min.) time intervals after administration of ciprofloxacin. Peak plasma concentrations of ciprofloxacin will be reached in 1-2 h. after oral administration in rats. Plasma was separated by centrifuging at 3000 rpm for 10 min and stored at –20ºC until analyzed for ciprofloxacin by microbiological assay, using *Escherichia coli* (ATCC 25922). Based on plasma concentrations, the pharmacokinetic parameters were determined by non compartmental methods. Detectable plasma concentrations of ciprofloxacin in rats persisted upto 6 h when ciprofloxacin was given alone whereas ciprofloxacin was detectable up to 8 h in quercetin pretreated rats. Prior administration of quercetin modified the kinetic profile of ciprofloxacin as evidenced by the significantly ($p<0.01$) higher area under curve (AUC), area under the first moment curve (AUMC) and there was significant ($p<0.01$) decrease in V_{dss} and Cl_B when compared to untreated group.

Key words: Ciprofloxacin, quercitin, pharmacokinetics, microbiological assay

1. Introduction

Antimicrobial fluoroquinolone agents are widely used, because of their broad spectrum and intense bactericidal activity. Ciprofloxacin is the most commonly used antimicrobial fluoroquinolone, both in humans and veterinary practice. Further, ciprofloxacin is an N-dealkylated pharmacologically active metabolite of enrofloxacin that was developed for exclusive use in veterinary practice. Ciprofloxacin is used orally or parentally in the treatment of infectious diseases affecting meninges, gastrointestinal tract, skin, respiratory system, bones and urogenital tract (Jones, 2002; Gasen *et al*., 2003; Wagenlehaner *et al*., 2006). It is also a preferred agent for the prevention and treatment of anthrax (Meyer hoff *et al*., 2004). It has an excellent activity against the enterobacteriaceae bacteria including many organisms that are resistant to penicillins, cephalosporins and aminoglycosides.

Pharmacokinetic behavior of ciprofloxacin was well characterized in calves, rabbits, ponies, sheep, rats, cow calves and buffalo calves (Nouws *et al*., 1988; Ramon *et al*., 1994; Dowling *et al*., 1995; Munoz *et al*., 1996; Kumar *et al*., 1997; Zhu *et al*., 1999a,b; Raina *et al*., 2000; Jayakumar *et al*., 2000). Intestine is considered an important pathway in elimination of fluoroquinolones including ciprofloxacin (Ramon *et al*., 1994). It was reported that intestinal elimination appears to be a leading cause for low bioavailability of ciprofloxacin in rabbits after oral administration (Sorgel *et al.*, 1989). Further, it was also reported that jejunum participates in the process of elimination of ciprofloxacin (Ramon *et al.*, 1994).

Transport of food ingredients and xenobiotics including drugs by ATP dependent transporters like P-glycoprotein (P_{GP}/MDR_1 / $ABCB_1$); multidrug resistance protein (MRP_S / $ABCC_S$) and breast cancer resistance protein (BCRP/ $ABCG_2$ /MXR /ABCP) in the intestine were well established in recent times (Brand *et al.*, 2006). These transporters are located specifically in the apical (intestinal luminal side) or basolateral (blood/plasma side) membrane of the enterocytes. BCRP is a member of the ATP binding cassette transporter G (Ejendal and Hrycyna, 2002; Doyle and Ross, 2003). BCRP is similar to half the duplicated P-gylcoprotein or multidrug resistance $protein_1$ molecule, hence, called as half transporter of ABCG sub family of ABC transporters (Doyle *et al.*, 1998; Kage *et al*., 2002). Further, Ando *et al*. (2007) reported that BCRP plays a significant role in the biliary excretion of fluoroquinolones and in the urinary excretion of ciprofloxacin (CPFX) and grepafloxacin (GPFX). Robey *et al.* (2009) reported that fluoroquinolones like ciprofloxacin, norfloxacin, ofloxacin are substrates of BCRP.

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Flavonoids are the most abundant polyphenolic compounds present in fruits, vegetables, and plant derived beverages such as grape fruit juice, tea and red wine (Dixon and Steele, 1999). Flavonoid mediated inhibition of ABC transporters including BCRP may affect the bioavailability of variety of xenobiotics and drugs (Szakacs *et al*., 2008). It was reported that flavonoid mediated inhibition of ABC transporters can be simple and safe approach in increasing the oral bioavailability of beneficial drugs that are the substrates of ABC transporters (Brand *et al.*, 2006). Curcumin, a flavonoid was reported to be an inhibitor of BCRP, increased the sensitivity of BCRP expressing cells to anticancer drugs (Chearwae *et al*., 2006). Quercetin, another flavonoid was also found to be the strongest inhibitor of $ABCG_2$ (BCRP) transport of SN-38 among natural flavonoids tested in the plasma membrane system and it was concluded that quercetin was considered to be good candidate for development of BCRP inhibitors (Yoshikawa *et al.*, 2004). Flavonoid consumption had significantly altered the pharmacokinetics and increased the toxicity or the antitumor action of ABCG2 substrate compounds (Imai, *et al*. 2004).This effect of flavonoids may be useful for increasing bioavailability of many clinically useful drugs like ciprofloxacin.

Keeping the above in view, the present study was carried out with the following objectives:

- To determine the plasma concentrations and pharmacokinetic parameters after single oral administration of ciprofloxacin in rats.
- To determine the plasma concentrations and pharmacokinetic parameters of ciprofloxacin after pretreatment with quercetin in rats.

2. Materials and Methods

2.1 Materials

Cifran 250 mg tablets from M/s Ranbaxy Laboratories, India were procured and used in the study for oral administration as a source of ciprofloxacin. For performing microbiological assay, technical grade ciprofloxacin generous gift from M/s PVS Laboratories (Vijayawada, India) was used. Quercetin of analytical grade compounds was obtained from M/s Sisco Research Laboratories. Heparin 20,000 IU/vial was obtained from M/s Loba Chemie. Xylazine was obtained from Indian Immunologicals Ltd., ketamine hydrochloride was obtained from Themis Medicare Ltd. *Escherichia coli* ATCC 25922 obtained from Post Graduate Institute of Medical Education, Chandigarh, was used in bioassay. All other chemicals and media used in the study were of analytical grade and were obtained from M/s Merck India and M/s Himedia, respectively.

2.2 Animals

Adult male rats of wistar albino strain were procured from Mahaveer Enterprises (Regd No: 146/1999/CPCSEA), Hyderabad. They were maintained under standard management and husbandry conditions with free access to feed and water. The experiments were approved by Institutional Animal Ethics Committee (IAEC), N.T.R College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh.

2.3 Microbiological assay

The concentrations of ciprofloxacin in the plasma were determined by an agar well diffusion microbiological assay, using *E.coli* (ATCC 25922) as the test organism. The diameters of the zones of bacterial inhibition were measured and converted to concentrations of ciprofloxacin equivalent by the use of a standard curve, calibrated by adding known amounts of pure ciprofloxacin to PBS (pH 7.4). Each test sample or standard was assayed in duplicate and the mean of the observations was determined. The limit of quantification of the assay was 0.06 μg/ml.

2.4 Pharmacokinetic studies

The present study was carried out in twelve male wistar rats, divided into two groups, six in each. The detailed pharmacokinetics of ciprofloxacin was investigated, based on plasma concentrations of ciprofloxacin obtained after single oral administration of ciprofloxacin (10 mg/kg) in control group, along with effect of prior administration of quercetin (20 mg/kg) on the plasma levels and pharmacokinetics of orally administered ciprofloxacin (10 mg/kg) in the respective groups. Pretreatment with quercetin was done in separate groups, 30 min. prior to the administration of ciprofloxacin. Rats were anaesthetized after oral administration of ciprofloxacin by xylazine (10-20 mg/kg) and ketamine (44-100 mg/kg) intraperitonealy. Supplemental doses of xylazine and ketamine combination were administered as required. Blood samples (200 μl) were collected into heparinised centrifuge tubes by tail vein clipping or jugular vein puncture as per convenience, prior to and at 0.33, 0.67,1, 1.5, 2, 4, 6, 8 and 12 h time intervals after ciprofloxacin administration. They were centrifuged at 3000 rpm for 10 min and plasma was harvested and kept at –20°C till analyzed for ciprofloxacin by microbiological assay.

2.5 Pharmacokinetic analysis

Plasma concentration versus time data of ciprofloxacin obtained in control and in pretreated groups, with quercetin in the present study was utilized for calculating various pharmacokinetic parameters (β, $t_{1/2\beta}$, $AUC_{0-\infty}$, $AUMC_{0-\infty}$, V_{dss}, Cl_B, and MRT) in rats by non compartmental methods (Milo Gibaldi and Perrier, 1982) and computer software (PK Solver Version 2.0 by Zhang Yang and Solver, 2010). The microbiological assay employed in the present study to measure the concentrations of ciprofloxacin in plasma, was also used previously by many authors to measure the concentrations of fluoroquinolones including ciprofloxacin in the plasma/serum of animals (Haritova *et al*., 2006; Akhtar *et al*., 2007; Raina *et al*., 2008; Muhammad and Muhammad, 2009). It was found that bioassay method correlates well with HPLC studies (Zuluaga *et al*., 2009, Abdelaziz *et al*., 2011, Manfio *et al*., 2013,).

Peak plasma ciprofloxacin concentration (C_{max}) and time to reach peak concentration (t_{max}) were calculated from actual plasma data of each rat. Elimination rate constant (β) of ciprofloxacin was calculated by least square regression analysis method. The area under the time plasma concentration (AUC_{0-t}) and of ciprofloxacin was calculated by linear trapezoidal rule, the ($AUC_{t-\infty}$) was calculated by dividing the last plasma concentration of the drug ($C_{t\,last}$) by elimination rate constant (β). $AUMC_{0-t}$ was also determined by linear trapezoidal rule. The elimination half life ($t_{1/2\beta}$) was calculated by using the formula $t_{1/2\beta} = 0.693/b$.

The mean residence time (MRT) was estimated from $MRT = AUMC_{0-\infty} / AUC_{0-\infty}$.

(V_{dss}) and (Cl_B) were calculated using equations V_{dss} = Dose x $AUMC_{0-\infty} / (AUC_{0-\infty})^2$

Cl_B = Dose/($AUC_{0-\infty}$)

2.6 Statistical analysis

All data were expressed as Mean±SEM ($SE_{\bar{X}}$). Differences in pharmacokinetic data between ciprofloxacin alone and pretreated quercetin groups were analysed for statistical significance, using unpaired student's'‘t’ test with Welch's correction using 'Instat' software. Difference of ($p<0.05$) and ($p<0.01$) were considered statistically significant.

3. Results

The mean plasma concentration of ciprofloxacin obtained in the present study is shown in (Table 1 and Figure 1).

Table 1: Effect of quercetin pretreatment on concentrations of ciprofloxacin in plasma (µg/ml) after single oral administration of ciprofloxacin at 10 mg/kg in group I and group II rats (n=6)

Time (h) Group-I	Ciprofloxacin	Quercetin+ Ciprofloxacin Group-II
0.33	0.75 ± 0.04	1.69 ± 0.056*
0.67	0.80 ± 0.02	1.93 ± 0.11*
1	1.03 ± 0.2	1.465 ± 0.17
1.5	0.57 ± 0.08	1.00 ± 0.058*
2	0.33 ± 0.04	0.645 ± 0.08*
4	0.17 ± 0.023	0.32 ± 0.03*
6	0.13 ± 0.004	0.17 ± 0.03
8	ND	0.12 ± 0.002

Values are expressed as Mean±SEM

*Significantly different ($p<0.01$) from respective normal values

ND means not determined

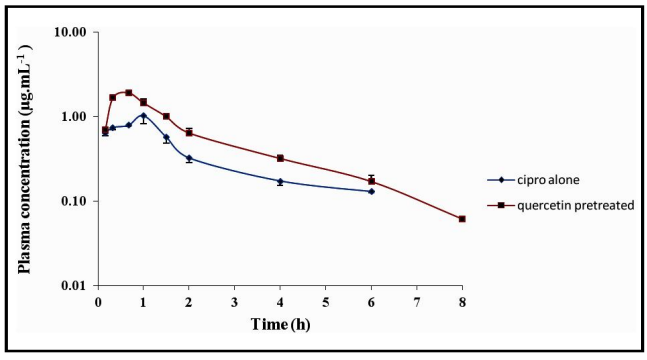

Figure 1: Semilogarithmic plot of ciprofloxacin concentrations in plasma versus time after single oral bolus administration of ciprofloxacin (10 mg/kg) in control (Blue plot) and quercetin pretreated (Red plot) in adult male wistar rats. Each point represents the Mean±SEM of six rats.

It was found that C_{max} of ciprofloxacin was significantly ($p<0.01$) higher in quercetin pretreated groups than in control. The plasma concentration of ciprofloxacin persisted up to 8 h in quercetin pretreated rats, while it was detected upto 6 h in the untreated group. The pharmacokinetic parameters of ciprofloxacin are presented in Table 2. Prior administration of quercetin modified the kinetic profile of ciprofloxacin as evidenced by the significantly ($p<0.01$) higher AUC, AUMC and there was significant ($p<0.01$) decrease in V_{dss} and Cl_B when compared to untreated group. Excipients used in the experiment have no role in microbiological assay.

Table 2: Pharmacokinetic parameters of ciprofloxacin in group I rats after single oral dose administration of ciprofloxacin (10 mg/kg) and in group ii rats after quercitin pretreatment (n=6).

Parameter	Unit	Ciprofloxacin alone Group-I	Quercetin + Ciprofloxacin Group-II
β	h^{-1}	0.404 ± 0.0499	0.298 ± 0.029
$t^{1}/_{2\beta}$	h	1.85 ± 0.221	2.00 ± 0.38
AUC_{0-t}	µg.h.mL^{-1}	2.03 ± 0.28	4.08 ± 0.29*
$AUC_{0-\infty}$	µg.h.mL^{-1}	2.38 ± 0.311	4.55 ± 0.27*
$AUC_{t-\infty} / AUC_{0-\infty}$	%	14.98 ± 1.717	10.48 ± 1.13
$AUMC_{0-t}$	µg.h^{2}.mL^{-1}	3.42 ± 0.72	8.06 ± 0.91*
$AUMC_{0-\infty}$	µg.h^{2}.mL^{-1}	6.26 ± 1.24	13.19 ± 1.14*
MRT	h	2.52 ± 0.2	2.88 ± 0.15
V_{dss}	L.kg^{-1}	10.98 ± 0.78	6.48 ± 0.51*
Cl_B	L.kg^{-1}.h^{-1}	4.56 ± 0.56	2.23 ± 0.14*
C_{max}	µg.mL^{-1}	1.115 ± 0.17	1.99 ± 0.1*
t_{max}	h	0.67 ± 0.122	0.72 ± 0.1

Values are expressed as Mean±SEM

*Significantly different ($p<0.01$) from respective normal values

4. Discussion

Although ciprofloxacin was reported to be well distributed with good bioavailability like other fluoroquinolone antimicrobial agents, active intestinal elimination of ciprofloxacin may be limiting its bioavailability *in vivo* (Ramon *et al*., 1994). In recent times, Robey *et al*. (2009) reported that fluoroquinolones like ciprofloxacin, norfloxacin, ofloxacin were substrates of breast cancer resistance protein (BCRP). BCRP is an ATP dependent efflux transporter, which is located specifically in the apical (intestinal luminal side) or baso lateral (blood/plasma) membrane of the enterocytes. It is reported that these drug transporters mediate cellular efflux of drugs that are substrates of BCRP in an active ATP dependent manner against concentration gradient and it was found to limit their oral bioavailability (Dietrich *et al*., 2003).

Kang *et al*. (2009) reported that natural compounds like flavonoids such as quercetin, curcumin, genistein, nariginin, *etc*., gluocosides such as sinomenine, alkaloids such as piperine, and saponins such as glycyrrhizin have demonstrated capacity to enhance the absorption and bioavailability of co-administrated drugs by inhibiting

efflux drug transport across the epithelia. It is suggested that modulation of BCRP mediated drug efflux using flavonoids can be a simple, relatively safe and promising approach.

Quercetin, a bioflavonoid found in leafy vegetables, apples, onions and berries has got many biological effects. Quercetin stimulated cellular accumulation of several BCRP substrates in BCRP over expressing cell lines, indicating inhibitory interaction with BCRP (Yoshikawa *et al*., 2004; Zhang *et al*., 2004).

Based on the above available data it can be interpretated that ciprofloxacin, like other fluoroquinolones is a BCRP substrate, whose bioavailability is being limited by drug efflux mediated by BCRP, an ATP dependent drug transporter. Hence, in the present study an attempt was made to use proven BCRP inhibiting flavonoids such as quercetin to enhance the plasma concentrations of ciprofloxacin upon oral administration in rats.

Upon single oral administration of ciprofloxacin (10 mg/kg) resulted in a peak plasma drug concentration (C_{max}) of 1.12±0.18 $\mu g.ml^{-1}$ which was significantly ($p<0.01$) lower than in quercetin pretreated groups 1.99±0.1 µg/ml. The $AUC_{0-\infty}$ of ciprofloxacin obtained in control 2.38±0.31 µg.h/ml is significantly ($p<0.01$) lower than in quercetin pretreated group 4.55±0.27 µg.h/ml.

Steady state volume of distribution V_{dss} of ciprofloxacin is significantly ($p<0.01$) lower in in quercetin pretreated 6.48±0.51 $l.kg^{-1}$ rats than in control group 10.98±0.784 $l.kg^{-1}$. There is a significant decrease in total body clearance Cl_B in quercetin pretreated rats than in ciprofloxacin alone group, indicating decreased clearance of ciprofloxacin.

Based on the above pharmacokinetic data, it can be understood that use of flavonoid, quercetin prior to the administration of ciprofloxacin sustained the maintenance of therapeutic concentrations beyond 8 h and enhanced the concentrations of ciprofloxacin in plasma (microbiological assay results are shown in Figure 1), which is required for maximal clinical efficacy of fluoroquinolones.

5. Conclusion

From the present study, it is evident that increased plasma concentrations of ciprofloxacin and longer persistence of the drug in the body in quercetin treated rats for 30 min prior to the administration of ciprofloxacin, which might be due to their inhibitory action on BCRP mediated drug efflux. However, to confirm the above interpretation, it is necessary to conduct experiments upon the role of quercetin over the regulation of BCRP mRNA expression along the intestine.

Conflict of interest

We declare that we have no conflict of interest.

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