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Modification of pharmacokinetics of norfloxacin following oral administration of curcumin in rabbits

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Investigation was carried out in adult New Zealand white rabbits to study the influence of curcumin pre-treatment on pharmacokinetic disposition of norfloxacin following single oral administration. Sixteen rabbits were divided into two groups of eight each consisting of either sex. Animals in group-I were administered norfloxacin (100 mg/kg body weight p.o), while animals in group-II received similar dose of norfloxacin after pre-treatment with curcumin (60 mg/kg body weight per day, 3 days, p.o). Blood samples were drawn from the marginal ear vein into heparin-coated vials at 0 (zero time), 5, 10, 15, 30 min and 1, 2, 4, 6, 12 and 24 h post-treatment. Plasma norfloxacin concentrations were determined by high performance liquid chromatography. The plasma concentration-time profile of norfloxacin was adequately described by a one-compartment open model. The pharmacokinetic data revealed that curcumin-treated animals had significantly ($p \leq 0.05$) higher area under the plasma concentration-time curve and area under the first moment of plasma drug concentration-time curve. Prior treatment of curcumin significantly ($p \leq 0.05$) increased elimination half-life and volume of distribution of norfloxacin. Further treatment with curcumin reduced loading and maintenance doses by 26% and 24% respectively.

Keywords: *curcuma longa*, norfloxacin, oral administration, pharmacokinetics, rabbits

Introduction

Norfloxacin is a member of the fluoroquinolone group of antimicrobial agents. It has a wide spectrum of activity, excellent tissue penetration and is rapidly bactericidal at low concentrations. Norfloxacin has a minimum inhibitory concentration (MIC_{90}) of 0.06, 0.12, 0.25 and 0.5 mg/mL for *Haemophilus influenzae, Escherichia coli, Enterobacter* spp. and *Klebsiella* spp., respectively [2]. This antibiotic shows promise as an antimicrobial agent for bacterial diseases of the respiratory, genito-urinary and gastro-intestinal tracts [22]. Encouraging results have been observed following the therapeutic use of norfloxacin in dogs suffering from hemorrhagic gastroenteritis caused by *E. coli, Salmonella* spp., and *Shigella* spp. [3], and norfloxacin has been successfully employed to treat genital tract infections caused by *Pseudomonas aeruginosa* in bulls [10]. The absolute bioavailability of norfloxacin in humans and in laboratory animals is reported to be 40% [15], while in most domestic species the *per-os* bioavailability varies between 30～40% [11].

Turmeric (*Curcuma longa*) is a medicinal plant extensively used in *Ayurveda, Unani* and *Siddha* medicine as a home remedy for various diseases. Curcumin, which is the active component of *Curcuma longa*, improves the *per-os* bioavailability of the immunosuppressive agent mylophenolic acid by inhibiting non-specific drug metabolizing enzymes [4]. Similarly, curcumin suppresses drug metabolizing enzymes (CYP3A4) in the liver [23] as well as inducing changes in the drug transporter P-*glycoprotein*, hence increasing the maximum absorption concentration (C_{max}) and area under the plasma concentration-time curve (AUC) of celiprolol and midazolam in rats [24]. With this background, the present study was undertaken to evaluate the influence of curcumin pre-treatment on the disposition kinetics of norfloxacin and to assess its impact on dosage regimen in rabbits.

Materials and Methods

The study was conducted in New Zealand white rabbits weighing 1.65 ± 0.22 kg, divided into two groups with eight rabbits in each group. The rabbits were acclimatized for three weeks to laboratory conditions before initiating

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the experiment. They were housed in individual cages and fed with antibiotic free diet. Feed and water were provided *ad libitum*. Feed was withheld for at least 6 ~ 8 h before and until 4 h after drug administration. Necessary approval from the Institutional Animal Ethics Committee was obtained to carry out the investigation.

Norfloxacin (Aravind Pharma, India) was dissolved in 0.1 N HCl to obtain a 3.33% solution (50 mg of norfloxacin in 1.5 mL 0.1 N HCl). The required amount of curcumin (Sigma-Aldrich, USA) was dissolved in a mixture of distilled water and Tween-20 at a 2 : 1 ratio restricting the total volume to 4 ~ 5 mL. Group-I rabbits (control) received norfloxacin at the rate of 100 mg/kg body weight as a single oral dose. The rabbits in group-II were administered a similar dose of norfloxacin after pre-treatment with curcumin (60 mg/kg body weight; p.o) for three days at an interval of 24 h. Blood samples (1.0 ~ 1.5 mL) were aseptically drawn from the marginal ear vein into heparin-coated tubes (Hi-Media, India) immediately before (0) at 5, 10, 15 and 30 min, and 1, 2, 4, 6, 8, 12 and 24 h after the administration of norfloxacin. Plasma samples were obtained by centrifugation of each blood sample (1,250 ×g, 10 min) and were stored at −20°C (for not more than 24 h) until being assayed.

Plasma norfloxacin concentrations were determined using high performance liquid chromatography (HPLC; Shimadzu, Japan). Dilutions of norfloxacin (E. Merck, India) ranging from 0.01 ~ 4 mg/mL were carried out with the mobile phase to obtain a standard curve. The HPLC system consisted of double pump (LC-20AT), rheodyne manual injector with 20 µL loop, dual wavelength ultraviolet detector (SPD-20A) and LC Solution software for data analysis. Chromatography was carried out using a reverse phase C_{18} column (250 × 4.5 mm, particle size 5 ± 0.3 µm, pore diameter 100 ± 10 A°; Phenomenax, USA) as a stationary phase. The mobile phase consisted of 0.1% v/v orthophosphoric acid (pH adjusted to 2.0) and acetonitrile mixed at a v/v ratio of 850 : 150. Chromatography was carried out at a flow rate of 1 mL/min at room temperature and the absorbance of norfloxacin at 275 nm was measured. The cleaned-up plasma samples [16] were analyzed for 8 min; there were no interfering peaks in the chromatogram at the retention time (R_t = 4.90 ± 0.14 min) of norfloxacin. The quantification limit was 0.015 µg/mL and the standard curve was linear in the range 0.015 ~ 4 µg/mL with a R^2 value of 0.999. Extraction recovery was determined to be 94.17% by comparing peak areas obtained for plasma-based standards and those obtained for mobile phase-based standards. The intra- and inter-day assay coefficients of variations were < 8.0%.

The plasma concentration-time profile of norfloxacin of each experimental animal was used to determine its pharmacokinetics. The pharmacokinetic data of norfloxacin was analyzed using the 'method of least square' and 'method of residual yields' [8]. The compartmental analysis of the data was undertaken using the mono-exponential equation:

$$C^t_p = Be^{-\beta t} - Ae^{-Kat}$$

where, C^t_p = plasma drug concentration, B is the zero-time intercept of regression line of elimination phase, A is the zero-time plasma drug concentration intercept of regression line of absorption phase, K_a is the absorption rate constant, β is the overall elimination rate constant, t is the time and e is the natural logarithm base.

The total AUC and area under the first moment of plasma drug concentration-time curve (AUMC) were calculated as described previously [18]. The volume of distribution ($V_{d(area)}$) and clearance from the body (Cl_B) were calculated as previously described [8] for a non-vascular route of administration.

The loading and maintenance dosage schedules were selected to maintain a MIC of 0.1, 0.5 and 1.0 µg/mL in plasma [12].

The difference between the means of the two treatments was determined by student's *t*-test [21] and the data were analyzed using GraphPad Instant software (GraphPad Software, USA).

Results

The mean plasma concentration of norfloxacin was significantly ($p \leq 0.05$) higher in curcumin pre-treated rabbits, although such effect was not observed during the entire period of absorption phase (Table 1, Fig. 1). The plasma concentration of norfloxacin persisted up to 24 h in

Table 1. Comparison of mean plasma levels of norfloxacin (mg/mL) at different time intervals following oral administration in control (Group-I) and curcumin treated (Group-II) rabbits

Time (h)	Group-I	Group-II
0.08	0.37 ± 0.05	0.50 ± 0.25*
0.16	0.46 ± 0.04	2.87 ± 0.17*
0.25	2.23 ± 0.08	1.19 ± 0.01*
0.50	1.50 ± 0.25	1.08 ± 0.03
1.00	0.62 ± 0.01	0.84 ± 0.01*
2.00	0.48 ± 0.03	0.59 ± 0.01*
4.00	0.41 ± 0.01	0.32 ± 0.03
6.00	0.37 ± 0.01	0.21 ± 0.06
8.00	0.17 ± 0.04	0.13 ± 0.01
12.00	0.01 ± 0.001	0.09 ± 0.01*
24.00	ND	0.07 ± 0.02

*$p \leq 0.05$, ND = Not detected. Data are presented as mean ± SE.

curcumin-treated rabbits, while it was detected up to 12 h in the untreated control group (Table 1). The absorption rate constant and absorption half-life revealed a significant ($p \leq 0.05$) change (Table 2). Prior administration of curcumin modified the kinetic profile of norfloxacin as evidenced by the higher AUC, AUMC and mean resident time. Prior administration of curcumin significantly ($p \leq 0.05$) reduced the elimination rate constant (β) and consequently increased the half-life of norfloxacin. Similarly, there was a significant increase in $V_{d(area)}$ of norfloxacin in curcumin-treated rabbits when compared to untreated controls (Table 2). Prior treatment with curcumin reduced both loading and maintenance doses up to 26.0% and 24.0%, respectively, at different norfloxacin MICs (Table 3).

Discussion

Norfloxacin has antimicrobial activity against a wide range of bacteria and is being effectively used to treat respiratory, urinary and gastro-intestinal tract infections in man and animals. Pharmacokinetic studies on norfloxacin in rabbits are limited [14,19]. The absorption of norfloxacin from gastrointestinal tract is limited [5,9]. Curcumin, a flavonoid isolated from *Curcuma longa*, improves the therapeutic concentrations of co-administered drugs [4,24]. With this background, the present study was undertaken to examine the influence of curcumin on the disposition profile of norfloxacin in rabbits after oral administration.

The disposition of norfloxacin after a single oral dose

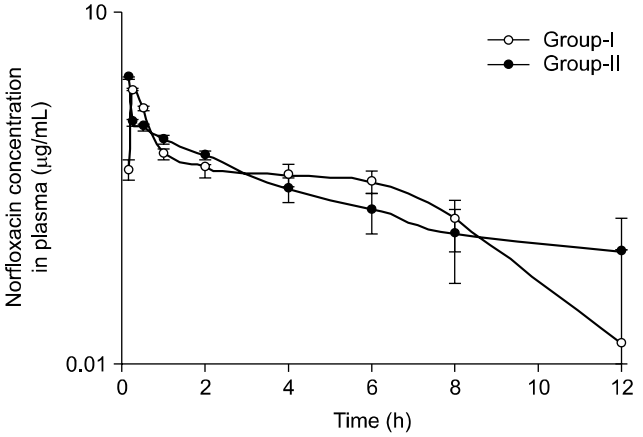

Fig. 1. Semilogarithmic plot of plasma concentration-time profile of norfloxacin in control (Group-I) and curcumin treated (Group-II) rabbits following single oral dose administration.

Table 2. Comparative pharmacokinetics of orally administered norfloxacin (100 mg/kg body weight) in control (Group-I) and curcumin treated (Group-II) rabbits

Parameter	Unit	Group-I	Group-II
K_a	/h	1.84 ± 0.03	2.59 ± 0.62*
A	µg/mL	1.95 ± 0.14	1.85 ± 1.15
β	/h	0.278 ± 0.01	0.231 ± 0.03*
B	µg/mL	1.04 ± 0.01	1.11 ± 0.02
$t_{½Ka}$	/h	0.35 ± 0.01	0.27 ± 0.06*
$t_{½β}$	/h	2.49 ± 0.13	2.96 ± 0.34*
AUC	µg/mL/h	2.67 ± 0.42	4.06 ± 1.24*
AUMC	µg/mL/h^2	13.40 ± 1.62	22.64 ± 6.34*
MRT	H	5.01 ± 0.19	5.60 ± 0.15*
$V_{d(area)}$	L/kg/h	5.69 ± 0.28	7.45 ± 1.70*
Cl_B	L/kg/h	1.49 ± 0.11	1.58 ± 0.03
t_d	H	27.72 ± 6.22	29.58 ± 3.84*

*$p \leq 0.05$. k_a: absorption rate constant, A: zero time plasma drug concentration intercept of regression line of absorption phase, β: overall elimination rate constant, B: zero time intercept of regression of elimination phase, $t_{½Ka}$: absorption half-life, $t_{½β}$: elimination half-life, AUC: area under the plasma concentration-time curve, AUMC: area under first moment of plasma drug concentration-time curve, MRT: mean residence time, $V_{d(area)}$: apparent volume of distribution, Cl_B: total body clearance of drug, t_d: total duration of pharmacological effect. Data are presented as mean ±SE.

Table 3. Dosage regimen of norfloxacin, calculated on the basis of pharmacokinetics values of obtained following oral administration of curcumin treated (Group-II) and control (Group-I) rabbits at various dosage intervals for microorganisms of different susceptibilities

Susceptibility of microorganisms (MIC)*	Dosage interval					
	Group-I			Group-II		
	6 h	8 h	12 h	6 h	8 h	12 h
0.1	3.01† (2.45)‡	5.25 (4.69)	15.99 (15.42)	2.97 (2.23)	4.73 (3.98)	11.91 (11.17)
0.5	15.08 (12.23)	26.29 (23.45)	79.96 (77.12)	14.89 (11.20)	23.64 (19.92)	59.56 (55.84)
1.0	30.16 (24.47)	52.59 (46.90)	159.92 (77.11)	29.79 (22.34)	47.29 (39.84)	119.13 (111.68)

*Values given are expressed as µg/mL. †Values given are expressed as mg/kg body weight. ‡Values given are loading doses and the values in parenthesis are maintenance doses.

(100 mg/kg body weight) was examined in rabbits with or without prior exposure to curcumin. A similar dose (*per os*) has been used to describe plasma and tissue concentration of norfloxacin in rabbits [19]. The observed plasma concentration-time profile of norfloxacin was best described by the one compartment open model. The plasma levels of norfloxacin (group-I) at different time intervals were comparable to previous studies in rabbits receiving a similar dose [19], however, the plasma half-life was relatively short [14]. The increased plasma levels of norfloxacin observed in the present study (group-II) may be due to the by-pass of glucuronidation process in the intestine since curcumin was reported to suppress UDP-glucuronyltransferase levels in intestine and hepatic tissue [4]. Furthermore, the ability of curcumin to suppress CYP3A4 drug metabolizing enzymes [23] might have delayed the excretion of norfloxacin. It is more likely that the increased absorption observed in the present study may have been due to the ability of curcumin to influence drug transporter protein (P-*gp*) in the intestine, as occurs with celiprolol [24]. Similarly, curcumin and gingerol (from ginger) were observed to inhibit P-*gp* mediated ^{3}H-digoxin transport in L-MDR 1 and caco-2 cells *in vitro* [23]. Furthermore, the modification of physiological activity in the gastrointestinal tract by curcumin [3,17] in the group-II rabbits might have contributed to the improved absorption of norfloxacin.

Norfloxacin undergoes extensive metabolism in the liver involving both Phase-I and Phase-II [1]. The significantly higher values of AUC, AUMC and mean residence time (MRT) observed in the present study might be attributable to the enhanced systemic availability of norfloxacin consequent to inhibition of enzymes mostly concerned with the hepatic metabolism of norfloxacin. Furthermore, in contrast to the fact that curcumin can induce hepatic glucuronyltransferase, its suppression at a higher dose cannot be ruled out. It is noteworthy that curcumin is itself metabolized through hepatocytes as glucuronides of tetrahydrocurcumin [13] and, therefore, the metabolism of norfloxacin may be delayed due to competition between two substrates.

The higher plasma elimination half life ($t_{1/2\beta}$) of 2.96 ± 0.34 h in the curcumin-treated group when compared to the control group could be due to prolonged persistence of the drug in the body due to inhibition of one or more enzyme(s) concerned with metabolism of norfloxacin. A significant amount of norfloxacin was excreted unchanged via renal mechanisms [15]. Therefore, it can be hypothesized that curcumin might have delayed the excretory mechanism of norfloxacin, since P-*gp* protein also exists in the proximal convoluted tubules.

From a practical point of view, a dosage regimen of 80 and 77 mg/kg of norfloxacin alone or 60 and 55 mg/kg of norfloxacin after curcumin pre-treatment as the loading and maintenance dose, respectively, at a 12 h interval adequately maintains optimal therapeutic concentration of 0.5 μg/mL plasma against resistant pathogens infecting rabbits. The reduction in the loading and maintenance doses indicates that prior administration of curcumin is of economic significance as well as being capable of reducing side effects, as a lesser amount of drug would be required. The bioenhancer nature of curcumin is comparable to piperine [20], an alkaloid obtained from *Piper longum*. Thus, bioenhancer properties of curcumin can be clinically exploited after appropriate dose titration studies.

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