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Interaction of vitamin B1 and phenylalanine on the pharmacokinetics of ciprofloxacin using bioassay in rabbits

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ABSTRACT

This pharmacokinetic study was carried out in rabbits following single oral administration of ciprofloxacin to study kinetic behaviour of ciprofloxacin in the presence of vitamin B1 and selected essential amino acid phenylalanine. Rabbits weighing 2-4 kg were randomly assigned to 5 groups of 6 each. Group 1 served as blank without any treatment. Group 2 served as ciprofloxacin control whereas Group 3, 4 and 5 rabbits were co-administered with thiamine (80 mg/kg), phenylalanine (48 mg/kg), combination of thiamine and phenylalanine along with ciprofloxacin (40 mg/kg) orally. Blood samples were collected at predetermined time intervals up to 24 h, and plasma was used for estimation of ciprofloxacin through bioassay. Results obtained from the experiment revealed that rabbits of Group 3, 4 and 5 showed improved levels of Cmax 4.74±0.17, 3.00±0.22, 2.18±0.12 µg/mL and AUC 20.30±5.19, 10.09±1.48, 12.85±1.34 µg.h/mL, respectively when compared to Cmax (2.10±0.14 µg/mL) and AUC (5.90±0.81 µg.h/mL) in Group II rabbits. PK-PD integration of the present study revealed that Cmax: MIC>8, AUC: MIC>125 in Group 3, 4 and 5 implying that the co-administration of vitamin B1 and phenylalanine and their combination improves the antibacterial activity and reduces the development of resistance at the selected dose of ciprofloxacin.

Keywords: Ciprofloxacin, Pharmacokinetics, Phenylalanine, Rabbits, Vitamin B1

The interplay of drugs continues to be a significant outcome of pharmacological interventions. Ciprofloxacin, is a second-generation fluoroquinolone derived from nalidixic acid with a broad range of applications in both human and veterinary medicine due to its ability to combat a wide spectrum of bacterial infections (Saadeh *et al*. 2019). The antibacterial activity of ciprofloxacin is produced by inhibiting the function of DNA gyrase and topoisomerase IV enzymes, which are essential for bacterial DNA replication and repair, thereby halting bacterial growth and reproduction. Ciprofloxacin is a concentration dependent bactericidal antibacterial agent (Sharma *et al*. 2017). Quinolones, particularly ciprofloxacin, have favourable pharmacokinetic characteristics in animals, and their apparent volume of distribution suggests significant tissue penetration (Shaheen *et al*. 2011). Although ciprofloxacin is widely distributed with good bioavailability as other fluoroquinolone antimicrobial agents, the active intestinal elimination of ciprofloxacin could potentially restrict its bioavailability *in vivo* (Devi *et al*. 2016).

Thiamine, also known as vitamin B1, belongs to the group of water-soluble B vitamins. It is naturally found in certain foods, added to some food products, and can be obtained through dietary supplements. In the United States and many other countries, thiamine fortification is common in breads, cereals, and infant formulas. Thiamine plays, a pivotal role in energy metabolism, growth, development and functioning of cells (Coates *et al*. 2010). Amprolium, a thiamine analogue, is employed for treating and preventing coccidiosis in poultry and rabbits. It is commonly incorporated into poultry feed as an additive, which could potentially interact with any administered drugs (El-sayed *et al*. 2014) thereby thiamine co-administered with ciprofloxacin may alter the kinetic behaviour of ciprofloxacin.

Phenylalanine is an essential amino acid that is important for various biological processes in all animals, including rabbits. The sources of phenylalanine include foods like wheat germ, oats, dairy products, and various meats. Phenylalanine is an essential amino acid and can be converted into tyrosine, but the reverse process is not possible which is further used in the production of catecholamines like dopamine and norepinephrine. Phenylalanine produces synergistic activity in combination with antibiotics such as amoxicillin, lincomycin, cefoxitin, bacitracin and vancomycin by improved area of zone of inhibition (Sen *et al*. 2019).

The antimicrobial characteristics of a group of quaternary

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ammonium compounds originating from phenylalanine, and the antimicrobial efficacy of these compounds was affected by the length of the alkyl chain (Lukac *et al*. 2010). Based on the available literature, it was found that there are no reports available on the drug interaction studies of thiamine and phenylalanine along with ciprofloxacin. Hence the present study was designed to study the kinetic behaviour of ciprofloxacin in the presence of thiamine, phenylalanine and their combination in rabbits.

MATERIALS AND METHODS

The present study was carried out to determine the pharmacokinetics and antibacterial activity of ciprofloxacin alone and in the presence of vitamin B1, selected essential amino acid phenylalanine in adult male New Zealand white rabbits procured from Mahaveer Enterprises, Hyderabad, Telangana (Reg. No 146/1999/CPCSEA). The experiment was conducted in the Department of Veterinary Pharmacology and Toxicology and Experimental Laboratory Animal House, N.T.R. College of Veterinary Science, Gannavaram, Andhra Pradesh. The protocol of experiment was approved by Institutional Animal Ethics Committee (vide No: 02/IAEC/NTR CVSc/2023), NTR College of Veterinary Science, Gannavaram.

Drugs and chemicals: The study used-Cifran 250 mg tablets; Ciprofloxacin [M/s Sisco Research Laboratories Pvt. Ltd. (SRL)]; Thiamine and phenylalanine [M/s Sisco Research Laboratories Pvt. Ltd. (SRL)]; Heparin 20,000 IU/vial (M/s Loba Chemie); and *Escherichia coli* MTCC 443.

Preparation of drug solutions: A 10% ciprofloxacin hydrochloride oral solution was prepared in normal saline (0.9% sodium chloride) and 1.6 g of thiamine was dissolved in 10 mL normal saline (0.9% sodium chloride) and thiamine suspension was made by trituration, the final concentration of the thiamine suspension was 160 mg/mL and 1.5 g of phenylalanine was dissolved in 10 mL normal saline (0.9% sodium chloride) and the final concentration of phenylalanine oral suspension was 150 mg/mL.

Experimental design: Adult male rabbits (New Zealand white) having body weight in the range of 2-4 kg were randomly assigned to 5 groups consisting of six rabbits in each group. Ciprofloxacin (40 mg/kg) was administered as a single oral bolus dose in all the groups. In addition, co-administration of thiamine (80 mg/kg), phenylalanine (48 mg/kg) and combination of both thiamine and phenylalanine orally prior to the administration of ciprofloxacin in group 3, 4, and 5 rabbits was done, respectively.

Microbiological assay: The concentrations of ciprofloxacin in the plasma was assessed through a microbiological assay employing agar well diffusion. *E. coli* (MTCC 443) served as the test organism. The area of the zone of inhibition of bacteria was measured and transformed into ciprofloxacin concentrations using a standard curve. This curve was established by introducing known quantities of pure ciprofloxacin to PBS (*p*H 7.4). Every test sample or standard underwent duplicate assays and the average of the results was calculated. The assay's quantification limit was 0.06 µg/ml.

Collection of blood samples: Blood samples (500 µl) were collected from the ear vein before ciprofloxacin administration and at regular time intervals of 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, and 24 h post ciprofloxacin administration. These samples were then centrifuged at 3000 rpm for 10 min, and the plasma obtained was stored at -20°C until analysis for ciprofloxacin using microbiological assay.

Pharmacokinetic analysis: Plasma concentration versus time data of ciprofloxacin obtained in control and in pretreated groups with thiamine and phenylalanine 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 rabbits by non-compartmental methods (Gibaldi and Perrier 1982) and computer software (PK functions 2.0). Peak plasma ciprofloxacin concentration (C_{max}) and time to reach peak concentration (t_{max}) were calculated from actual plasma data of each rabbit. Elimination rate constant (β) of ciprofloxacin which was calculated by least square regression analysis method. The area under the time plasma concentration (AUC_{0-1}) 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:

$$t_{1/2\beta}=0.693/\beta$$

The mean residence time (MRT) was estimated from $MRT= AUMC_{0-\infty}/AUC_{0-\infty}$ (V_{dss}) and (Cl_B) were calculated using equations:

$$V_{dss} = Dose \times AUMC_{0-\infty}/ (AUC_{0-\infty})^2$$

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

$$C_p^{\,t} = C_p^{\,0}.e^{-\beta t}$$

Statistical analysis: The plasma concentrations of ciprofloxacin were expressed as Mean±SEM. Differences in pharmacokinetic data between ciprofloxacin alone and co-administration of ciprofloxacin with thiamine and phenylalanine groups were analyzed for statistical significance using the software SPSS 17.0 version. Means were compared and the statistical significance of the differences between groups was assessed using one-way analysis of variance followed by Duncan's multiple range test. Difference of ($p<0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

The mean plasma concentration of ciprofloxacin obtained in this investigation is presented in Table 1 and Fig. 1. Following a single oral administration of ciprofloxacin at 40 mg/kg, the peak plasma drug concentration (C_{max}) was estimated. C_{max} was increased to 4.74±0.17 µg/mL in thiamine co-administered group and it was improved to 3.00±0.22 µg/mL in phenylalanine

Table 1. Effect of co-administration of thiamine, phenylalanine and combination of both thiamine and phenylalanine on concentrations of ciprofloxacin in plasma (µg/ml) after single oral administration of ciprofloxacin at 40 mg/kg in group 2, group 3, group 4 and group 5 rabbits (n=6)

Time (h)	Group 2 (Ciprofloxacin)	Group 3 (Thiamine+ Ciprofloxacin)	Group 4 (Phenylalanine +Ciprofloxacin)	Group 5 (Thiamine+ Phenylalanine+ Ciprofloxacin)
0.25	1.51±0.14	3.42±0.48**	2.66±0.37*	1.90±0.20
0.5	2.00±0.14	3.47±0.19**	2.74±0.15**	2.07±0.10
0.75	1.37±0.24	4.54±0.28**	2.33±0.30*	2.15±0.14*
1	1.23±0.24	3.30±0.25**	2.43±0.26**	1.57±0.07*
2	0.98±0.15	2.80±0.47**	1.62±0.29	1.33±0.13*
4	0.60±0.10	1.66±0.39b**	0.94±0.19	1.51±0.18*
6	0.25±0.06	1.11±0.34**	0.50±0.09	0.82±0.17
8	0.13±0.05	0.77±0.28*	0.26±0.05	0.50±0.11
12	ND	0.39±0.19*	0.08±0.02	0.29±0.07

Values are expressed as Mean±SEM. *, Significantly different ($p<0.05$) from respective ciprofloxacin alone values; ND, not determined.

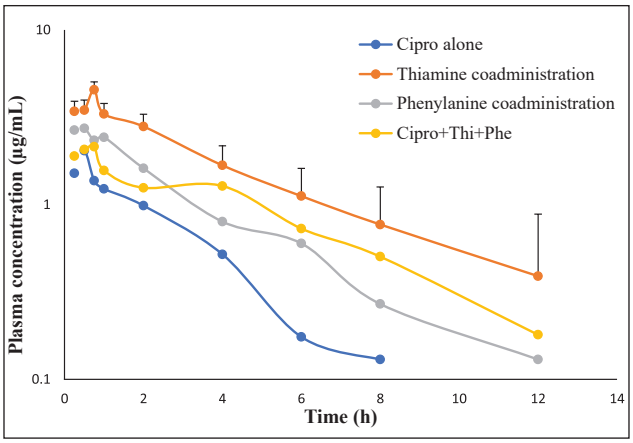

Fig. 1. Comparative semilogarithmic plot of ciprofloxacin concentrations versus time in Group 2, Group 3, Group 4 and Group 5 rabbits (Each point represents the Mean±SEM of six rabbits).

co-administered with ciprofloxacin in rabbits when compared to group 2 rabbits which received only ciprofloxacin (2.10±0.14 µg/mL) at $p<0.05$ level of significance. Relatively lower C_{max} values of 1.49 µg/mL, 1.46 µg/mL, and 1.24 µg/mL were reported by Bashir *et al*. (2010), Al-Ghazawi *et al*. (2012), Saadeh *et al*. (2019), respectively in rabbits upon oral administration of ciprofloxacin. Higher C_{max} values of 3.45±0.34 µg/mL was reported by Ukpo *et al*. (2017) in rabbits. The variations in C_{max} may be due to different doses of ciprofloxacin and its behaviour in different species.

The bioavailability (AUC_{0-t}) of group 2 rabbits was 5.64±0.66 µg.h/mL which was significantly increased in thiamine co-administered rabbits 17.80±3.59 µg.h/mL at $p<0.05$ level of significance (Table 2). This may be attributed to the inhibitory activity of thiamine on hepatic microsomal enzymes (Grosse *et al*. 1971). As ciprofloxacin is metabolized through CYP 1A2 microsomal enzyme, inhibition of the microsomal enzyme activity by the thiamine leads to a reduced rate of metabolism of ciprofloxacin which leads to an increase in the bioavailability as well as duration of action of drug up to 12 h when compared to the ciprofloxacin control which is only 8 h (Grosse *et al*. 1971).

Steady state volume of distribution (V_{dss}) of ciprofloxacin is considerably lower in thiamine, phenylalanine and their combination (0.23±0.01 L/kg, 0.33±0.03 L/kg, 0.42±0.03 L/kg, respectively) than in the ciprofloxacin control group (0.53±0.04 L/kg). Lower V_{dss} values obtained in the present study suggest that ciprofloxacin is not stored in the tissues of rabbits. There is a substantial decrease in total body clearance Cl_B in thiamine (group 3), phenylalanine (group 4) and their combination group (group 5) compared to the ciprofloxacin alone group (group 2), indicating lower ciprofloxacin clearance. This may be attributed to the intestinal tract as an important pathway in eliminating ciprofloxacin and other fluoroquinolones besides glomerular filtration (Ramon *et al*. 1994). The difference in clearance among the species may be due to varied metabolism and elimination mechanisms.

The study revealed a significant ($p<0.05$) increase in the maximum plasma concentration (C_{max}) of ciprofloxacin

Table 2. Pharmacokinetic parameters of ciprofloxacin in group 2 rabbits after single oral dose administration of ciprofloxacin (40 mg/kg) and in group 3, 4 and 5 rabbits after thiamine, phenylalanine and their combination coadministration (n=6)

Parameter	Unit	Ciprofloxacin alone	Thiamine+ Ciprofloxacin
β	1/h	0.33±0.05	0.32±0.10
$t_{1/2\beta}$	H	1.99±0.37	2.95±0.62
AUC_{0-t}	µg.h/mL	5.64±0.66	17.80±3.59**
$AUC_{0-\infty}$	µg.h/mL	5.90±0.81	20.30±5.19*
$AUC_{t-\infty}$/ $AUC_{0-\infty}$	%	3.18±1.94	7.95±3.46*
$AUMC_{0-t}$	µg.h²/mL	16.16±3.53	66.63±20.21*
$AUMC_{0-\infty}$	µg.h²/mL	20.31±3.66	114.18±53.72*
MRT	h	3.12±0.54	4.46±0.91*
V_{dss}	L/kg	0.53±0.04	0.23±0.01**
Cl_B	L/kg/h	7.45±1.02	2.62±0.63**
C_{max}	µg/mL	2.10±0.14	4.74±0.17**
t_{max}	h	0.54±0.04	0.91±0.22

Values are expressed as Mean±SEM. *, Significantly different ($p<0.05$) from respective ciprofloxacin alone administered values.

Table 3. pharmacokinetic parameters of ciprofloxacin in group 2 rabbits after single oral dose administration of ciprofloxacin (40 mg/kg) and in group 3, 4 and 5 rabbits after thiamine, phenylalanine and their combination coadministration (n=6).

Parameter	Unit	Phenylalanine+ Ciprofloxacin	Thiamine+ Phenylalanine + Ciprofloxacin
β	1/h	0.42±0.12	0.28±0.08
$t_{1/2\beta}$	h	2.10±0.32	3.25±0.52
AUC_{0-t}	µg.h/mL	9.76±1.44	11.24±0.93
$AUC_{0-\infty}$	µg.h/mL	10.09±1.48	12.85±1.34
$AUC_{t-\infty}$/ $AUC_{0-\infty}$	%	2.92±0.88	11.20±3.04*
$AUMC_{0-t}$	µg.h^2/mL	29.53±5.19	46.65±7.27*
$AUMC_{0-\infty}$	µg.h^2/mL	34.60±6.25	75.34±15.72*
MRT	h	3.20±0.41	5.49±0.80*
V_{dss}	L/kg	0.33±0.03**	0.42±0.03*
Cl_B	L/kg/h	5.05±1.53	3.32±0.41**
C_{max}	µg/mL	3.00±0.22**	2.18±0.12
t_{max}	h	0.58±0.13	1.30±0.54

Values are expressed as Mean±SEM. *Significantly different ($p<0.05$) from respective ciprofloxacin alone administered values.

when co-administered with thiamine and phenylalanine, as compared to the ciprofloxacin control group. In rabbits, the presence of thiamine, phenylalanine, and their combination extended the detectable plasma concentration of ciprofloxacin to 12 h, while it was detected up to 8 h in the ciprofloxacin control group. The pharmacokinetic parameters of ciprofloxacin are presented in Tables 2 and 3. Co-administration of thiamine, phenylalanine, and their combination with ciprofloxacin resulted in a modification of the kinetic profile, indicated by significantly higher ($p<0.05$) values for AUC and AUMC. Additionally, there was a notable decrease ($p<0.05$) in V_{dss} and Cl_B compared to the group administered with ciprofloxacin alone. It's important to note that the excipients used in the experiment did not play a role in the microbiological assay.

The conclusions drawn from the study are: Thiamine co-administered with ciprofloxacin improved peak plasma concentrations, bioavailability and duration of action of ciprofloxacin in rabbits; Phenylalanine co-administration with ciprofloxacin improved the C_{max} and bioavailability by 70% and 58.47% in rabbits; the combination of thiamine and phenylalanine co-administration improved the bioavailability 45.91% and C_{max} 96.33%, MRT 56.83%. PK/PD integration revealed that combination of thiamine + ciprofloxacin (group 3) and thiamine + phenylalanine + ciprofloxacin (group 5) improved the antibacterial activity and reduced the development of resistance which may be useful strategy to improve the efficacy of ciprofloxacin and to combat the resistance. Upon all the tested combinations, thiamine with ciprofloxacin (group 3) offered better antibacterial activity when compared to phenylalanine and combination of phenylalanine with thiamine.

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