J. Vet. Sci. (2005), **6**(3), 197–200

JOURNAL OF
Veterinary
Science

Alteration of pharmacokinetics of oxytetracycline following oral administration of *Piper longum* in hens

M. Singh[1], C. Varshneya[1,*], R.S. Telang[1], A.K. Srivastava[2]

[1]*Department of Pharmacology and Toxicology, College of Veterinary & Animal Sciences, CSK Himachal Pradesh Agricultural University, Palampur-176 062, Himachal Pradesh, India*
[2]*Faculty of Veterinary and Animal Sciences, Sher-e-Kashmir University of Agricultural Science & Technology, R. S. Pura, Jammu-181102, India*

The pharmacokinetic profile of orally administered oxytetracycline (10 mg/kg body weight) was studied 7 days post oral treatment of *Piper longum* (15 mg equivalent/kg) in White Leghorn hens (2-2.8 kg). On the day 8, oxytetracycline (OTC) was administered orally and blood samples were collected from the wing vein in heparinised vials for plasma separation at 0 (pre-treatment), 15, 30, 60, 120, 240, 360, 480 and 600 minutes post OTC administration. Plasma OTC concentrations were determined by microbial assay technique using *Bacillus cereus* var. *mycoides* (ATCC 11778) as test organism. The plasma levels of OTC against time were adequately described by one compartment open model. The pharmacokinetic data revealed that *P. longum* treated animals had significantly higher area under curve (AUC), area under the first moment of plasma drug concentration-time curve (AUMC) and mean residential time (MRT). Prior treatment of *P. longum* significantly reduced elimination rate constant (β) and increased elimination half life ($t_{½\beta}$). The total body clearance (Cl_B) reduced by 21% whereas total duration of pharmacological effect (t_d) increased by 29%. The treatment with *P. longum* reduced loading and maintenance dose by 33.3 and 39%, respectively.

Key words: oral administration, oxytetracycline, pharmacokinetics, *Piper longum*, white Leghorn

Introduction

Oxytetracycline has wide scope in the treatment of various bacterial, rickettsial and mycoplasmal infections of poultry and other avian species. Notwithstanding its broad spectrum of activity, its therapeutic effectiveness is marred by its poor bioavailability by oral route [6].

Absorption of tetracyclines mostly takes place from stomach and upper part of small intestine. Most of the tetracyclines are incompletely and irregularly absorbed from gastrointestinal tract and only a small proportion of administered dose appears in the circulating blood and tissue at any given time [19].

Long pepper (*Piper longum*) is widely used in the Indian System of Medicine (Ayurvedic System) along with black piper and ginger to enhance the therapeutic efficacy of the concurrently administered drugs. The pretreatment of *P. longum* has been reported to increase the rate and extent of absorption of some of the drugs widely used in human medicine which are moderately absorbed on their own by enhancement of blood supply through vasodilatation [1,4], inhibition of their metabolism [5] or inhibition of renal clearance [5]. The present studies were therefore, undertaken to study whether *P. longum* pretreatment enhances the bioavailability of oxytetracycline administered orally in white Leghorn hens.

Materials and Methods

The study was conducted on poultry birds divided into two groups with 8 birds in each group weighing 2-2.8 kg. They were kept under close observation for one week before the commencement of experiment to enable them to acclimatize in the new environment. They were housed on deep litter and were fed an antibiotic free diet. Feed and water were provided *ad libitum*. The birds in Group I were administered oxytetracycline (Terramycin; Pfizer, India) orally at the dose rate of 10 mg/kg. The birds in Group II were fed *P. longum* at the dose rate of 15 mg piperine equivalent per kg/body wt for seven days. On day 8, oxytetracycline was administered orally as in Group I.

Blood samples of the animals of both the groups were collected from the wing vein in heparinized vials at time intervals of 0, 15, 30, 60, 120, 240, 360, 480 and 600 min.

*Corresponding author
Tel: +91-1894-230368; Fax: +91-1894-230511, +91-1894-230327
E-mail: cvarshneya@hotmail.com

The blood samples collected were centrifuged at 3,000 rpm for 10 minutes for plasma separation. The samples were frozen at –20°C till assayed.

P. longum was standardized for the presence of piperine content in it by spectrophotometric method developed at Research and Development Laboratory, Indian Herbs, Saharanpur [8].

The concentration of oxytetracycline in plasma was determined by using microbial assay technique using *Bacillus cereus* var *mycoides* (ATCC 11778) as the test microorganism [2].

The plasma concentration-time profile of oxytetracycline for each bird was used to determine its pharmacokinetics. Different pharmacokinetic parameters were analyzed using "method of least square" and "method of residual yields" as described by Gibaldi and Perrier [9]. The plasma concentration-time profile of oxytetracycline of each animal was used to determine pharmacokinetics.

Estimation of the areas under the concentration-time curve (AUC) and the area under the first moment of plasma drug concentration-time curve (AUMC) were based on trapezoidal rule [9]. The compartmental analysis was done using the mono-exponential equation:

$$Cp^{(t)} = Be^{-\beta t} - Ae^{-ka\,t},$$

where Cp is the 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 dosage regimen for maintaining MIC of 0.5, 1, 2, 3 and 4 µg/ml at the dosage interval of 6, 8 and 12 h was derived as per the method mentioned by Notari [12].

Results

The results of the study revealed that mean plasma concentration of oxytetracycline were significantly higher in *P. longum* treated birds during its entire absorption phase (Table 1, Fig. 1). The absorption rate constant and absorption half life, however, revealed a non significant change ($p > 0.05$). Further, findings of the present study indicated that prior administration of *P. longum* modified the kinetic profile of oxytetracycline (Table 2) as evidenced by higher area under curve (AUC), area under the first moment of plasma drug concentration time curve (AUMC), mean residence time (MRT) and total duration of pharmacological action (td).

Prior administration of *P. longum* significantly reduced the elimination rate constant (β) and subsequently increased the plasma half life of oxytetracycline (Table 2). Total body clearance also confirmed a similar trend in *P. longum* treated birds. There was significant reduction ($p < 0.01$) of about 21% in total body clearance as compared to control birds.

Table 1. Comparison of mean plasma levels of oxytetracycline (µg/ml) at different time intervals following oral administration in control and *Piper longum* treated birds (mean ± SE)

Time (min)	Control	Treated
15	0.23 ± 0.024	0.30 ± 0.022*
30	0.34 ± 0.025	0.45 ± 0.029**
60	0.72 ± 0.051	0.77 ± 0.046
120	0.58 ± 0.037	0.63 ± 0.030
240	0.37 ± 0.015	0.47 ± 0.034**
360	0.26 ± 0.017	0.38 ± 0.021****
480	0.23 ± 0.015	0.31 ± 0.019***
600	0.19 ± 0.005	0.25 ± 0.015***

*$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, ****$p < 0.001$

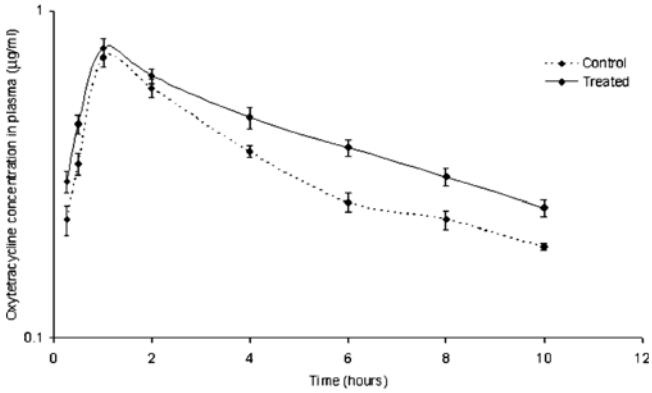

Fig. 1. Semilogarithmic plot of plasma concentration-time profile of oxytetracycline in *Piper longum* treated poultry following a single oral dose.

Computation of loading and maintenance dose revealed that prior treatment of *P. longum* reduced both loading and maintenance dose upto the extent of 33.26 and 39.0%, respectively at different minimum inhibitory concentrations of oxytetracycline (Table 3).

Discussion

Oxytetracycline has high bacteriostatic activity against a wide range of gram positive and gram negative microorganisms and is being effectively and successfully employed in the treatment of many bacterial infections in man and animals [14,15,18]. Absorption of oxytetracycline from gastrointestinal tract has been found to be inadequate and irregular. Only 60 % of the oral dose is absorbed. Antibiotic levels in blood and target organ directly affect the therapeutic efficacy of antibiotics. Piperine, an amide alkaloid isolated from different species of Piper has been reported to enhance the bioavailability of co-administered drugs [4,7,11,22]. So the study was undertaken to observe the effect of piperine on the pharmacokinetics of oxytetracycline.

Table 2. Comparative pharmacokinetics of oxytetracycline administered orally (10 mg/kg) in control and *Piper longum* treated birds (mean ± SE)

Parameters†	Unit	Control	Treated
K_a	/h	2.733 ± 0.639	2.105 ± 0.527
A	µg/ml	1.817 ± 0.559	1.033 ± 0.364
β	/h	0.147 ± 0.011	0.112 ± 0.0066*
B	µg/ml	0.794 ± 0.063	0.761 ± 0.049
$t_{1/2}$ K_a	h	0.351 ± 0.071	0.450 ± 0.078
$t_{1/2\beta}$	h	4.934 ± 0.422	6.370 ± 0.438**
AUC	µg/ml/h	5.055 ± 0.689	6.417 ± 0.371*
AUMC	µg/ml/h^2	43.62 ± 11.15	63.58 ± 7.042*
MRT	h	7.98 ± 0.744	9.77 ± 0.644**
t_d	h	16.38 ± 1.4	21.142 ± 1.453**
Cl_B	ml/kg/h	184.63 ± 7.7	145.97 ± 14.42**

†Pharmacokinetic parameters are as described by Gibaldi and Perrier [9]. K_a: absorption rate constant, A: zero time plasma drug concentration intercept of regression line of absorption phase, β: overall ellmination rate constant, B: zero time intercept of regression line of elimination phase, $t_{1/2}$ K_a: absorption half life, $t_{1/2\beta}$: ellmanation half life, AUC: area under curve, AUMC: area under first moment of plasma drug concentration-time curve, MRT: mean residential time, t_d: total duration of pharmacological effect. Cl_B: total body clearance.
*$p < 0.01$, **$p < 0.05$.

Table 3. Dosage regimen of oxytetracycline, calculated on the basis of pharmacokinetic values obtained following oral administration of *Piper longum* in poultry birds at various dosage intervals for microorganisms of different susceptibilities

Microorganism susceptibility (MIC)*	Dosage interval		
	6 h	8 h	12 h
0.5*	1.56† (0.91)‡	2.09 (1.45)	3.77 (3.12)
1	3.12 (1.83)	4.19 (2.90)	7.54 (6.25)
2	6.25 (3.66)	8.38 (5.79)	15.08 (12.49)
3	9.37 (5.49)	12.57 (8.69)	22.62 (18.74)
4	12.49 (7.32)	16.76 (11.58)	30.16 (24.98)

*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.

The experimental data of observed plasma concentrations revealed that pharmacokinetics of oxytetracycline in poultry birds by oral route was adequately described by one compartment open model. This model has also been used by various workers to describe the pharmacokinetics of oxytetracycline after extravascular administration in various animals [13,20]. The findings of the present study further revealed that mean plasma concentrations of oxytetracycline were higher in *P. longum* treated birds during its entire absorption phase. The increased plasma concentration of oxytetracycline during absorption could be due to enhanced blood supply in enteric vessels as a result of local vasodilatation [1,3]. The higher values of AUC, AUMC and MRT in piperine treated birds were indicative of enhanced systemic availability of oxytetracycline and suppression of drug metabolizing activities. Piperine, the active principle of *P. longum* has been shown to inhibit drug metabolizing activities [17]. Besides, piperine treatment has been shown to enhance blood levels of many chemotherapeutic agents including sulphadiazine, pyrazinamide, rifampin, isoniazid in human volunteers [21]. Piperine treatment has also been known to increase plasma levels of nimesulide [11] and indomethacin [10].

A reduction in the loading and maintenance dose clearly indicates interactive pharmacokinetics action of *P. longum* and oxytetracycline. It is, therefore, imperative to assume that prior administration of *P. longum* increases total duration of antimicrobial action and subsequently enhances therapeutic efficacy of oxytetracycline in poultry birds. The reduction in loading and maintenance dose will be of immense economic significance. This would also reduce the subsequent side-effects as lesser amount of drug would be required to achieve desirable therapeutic efficacy.

Acknowledgments

We express our thanks to the Dean, College of Veterinary & Animal Sciences, CSKHPKV, Palampur, for providing necessary facilities to carry out the present investigation.

References

1. **Annamalai AR, Manavalan R.** Effects of Trikatu and its individual components and piperine on gastrointestinal tract: Trikatu - A bioavailability enhancer. Indian Drugs 1989, **27**, 595-604.
2. **Arret B, Johnson DP, Krishbaum A.** Outline of details for microbiological assay of antibiotics. Second revision. J Pharm Sci 1971, **60**, 1689-1694.
3. **Atal CK, Dubey RK, Singh J.** Biochemical basis of enhanced drugs bioavailability by piperine: evidence that piperine is a potent inhibitor of mono oxygenase system. Ind J Pharm 1984, **16**, 52.
4. **Atal CK, Manavalan R, Nighojkar R, Sareen AN, Gupta OP.** Studies on *Piper chaba* as a bioavailable agent. Indian Drugs 1980, **17**, 266-268.
5. **Atal CK.** A breakthrough in drug bioavailability - A clue from age old wisdom of Ayurveda. Indian Drug Manufacturers' Assoc Bull 1979, **10**, 483-484.
6. **Atef M, El-Gendi AYI, Youssef SAH, Amer AMM.** Kinetic disposition, systemic bioavailability and tissue distribution of Oxytetracycline in chickens. Arch Geflügelk 1986, **50**, 144-148.
7. **Bano G, Amla V, Raina RK, Zutshi V, Chopra CL.** The effect of piperine on pharmacokinetics of Phenytoin in Healthy Volunteers. Planta Med, 1987, 53, 568-569.
8. **Chauhan SK, Kimothi GP, Singh BP, Agarwal S.** A spectrophotometric method to estimate piperine in Piper species. Ancient Sci Life 1998, **18**, 84-87.

9. **Gibaldi M, Perrier D.** Pharmacokinetics. 2nd ed. pp. 45-109, Marcel-Dekker, New York, 1982.
10. **Karan RS, Bhargava VK, Garg SK.** Effect of Trikatu on the pharmacokinetic profile of Indomethacin in rabbits. Indian J Pharm 1999, **31**, 160-161.
11. **Mathur P, Velpandium T, Sengupta S, Gupta SK.** Effect of piperine on analgesic activity of Nimesulide: A possible pharmacokinetic interaction. Indian J Pharm 1998, **30**, 204.
12. **Notari RE.** Biopharmaceutics and Clinical Pharmacoceutics: An Introduction. 4 th ed. pp. 221-270, Marcel-Dekker, New York, 1987.
13. **Prasad S, Jayachandran C, Singh MK.** Pharmacokinetics of oxytetracycline in dairy cows. Indian J Ani Sci 1987, **57**, 106-110.
14. **Prescott JF, Baggot JD.** Antimicrobial Therapy in Veterinary Medicine. pp. 193-204, Blackwell, Malden, 1988.
15. **Rang HP, Dale MM, Ritter JM.** Pharmacology. p. 696, Churchill Livingstone, Edinburgh, 2000.
16. **Reen RK, Singh J.** In vitro and in vivo inhibition of pulmonary cytochrome P450 activities by piperine, a major ingredient of Piper species. Indian J Expl Biol 1991, **29**, 568-573.
17. **Reen RK, Wiebel FJ, Singh J, Singh J**. Piperine inhibits aflatoxin B1 induced cytotoxicity and genotoxicity in V79 Chinese hamster cells genetically engineered to express rat cytochrome P450 2B1. J Ethnopharmacol 1997, **58**, 165-173.
18. **Skeeles JK, Swafford WS, Wages DP, Hellwig HM, Slavik MF, Beasley JN, Houghton GE, Blore PJ, Crawford D.** Studies on the use of a long acting oxytetracycline in turkeys: Efficacy against experimental infection with *Alcaligenes faecalis* and *Pasteurella multocida*. Avian Dis 1984, **24**, 1126-1130.
19. **Tisch D E, Cull K M, Dickison HL.** Antibiotics Annual, p. 668, Medical Encyclopedia, New York, 1954/1955.
20. **Toutain PL, Raynaud JP.** Pharmacokinetics of oxytetracycline in young cattle: comparison of conventional vs long-acting formulations. Am J Vet Res 1983, **44**, 1203-1209.
21. **Zutshi U.** Herbals as enhancer of bioavailability of antimicrobials. Indian J Pharma 1985, **17**, 120-127.
22. **Zutshi U, Zutshi RK, Singh R, Johri RK, Dhar SK, Rizvi NA.** Influence of piperine on the bioavailability pattern of antitubercular drugs. Indian J Pharma, 1986, **18**, 35.