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Pharmacoknetic Study of Diclofenac and Its Interaction with Enrofloxacin in Buffalo Calves

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Abstract

A comparative pharmacokinetic study of diclofenac (1 mg/kg, i.v.) when given alone or in combination with enrofloxacin (4 mg/kg, i.v.) in five buffalo calves was carried out by using HPLC. The study revealed that the plasma concentrations of diclofenac were significantly lower ($p<0.05$) in combined administration of diclofenac with enrofloxacin (0.042 to 3 h), whereas significantly higher ($p<0.05$) levels of plasma drug concentrations were observed in later period (8 to 24 h). In urine, significantly lower ($p<0.05$) drug concentrations of diclofenac were observed from 0.167 to 1.5 h, whereas significantly higher ($p<0.01$) urine drug concentrations were observed in later period (4 to 48 h) when diclofenac was given in combination with enrofloxacin as compared to when diclofenac was given alone. Various kinetic parameters like A, C_p^o and β were significantly lower ($p<0.05$) whereas $t_{1/2}\beta$, AUMC, MRT and various volume of distribution (Vd_C, Vd_B, Vd_{area} and Vd_{SS}) were significantly higher in combined administration of diclofenac with enrofloxacin as compared to when diclofenac was given alone ($p<0.05$).

Key Words: kinetics, diclofenac, interaction, enrofloxacin, buffalo calves

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are usually combined with antimicrobial agents to treat various systemic infections accompanied by fever and other inflammatory conditions.

Diclofenac is a potent NSAID with good analgesic, antipyretic and uricosuric properties. It produces its effects by irreversibly inhibiting the cyclooxygenase pathway of prostaglandin synthesis, which is the most common mediator of pain, inflammation and pyrexia. It is used in degenerative diseases, rheumatoid arthritis, ankolysing spondylitis and allied conditions [5].

Enrofloxacin, a recent fluoroquinolone antimicrobial belonging to carboxylic acid derivative was developed exclusively for veterinary use [1, 6]. It possesses a broad spectrum of activity against gram negative bacteria [15] and also against gram positive bacteria as well as mycoplasma [3]. In different species of animals, enrofloxacin is de-ethylated to ciprofloxacin [8,18] which is a potent antimicrobial agent used in human medicine [4]. Both enrofloxacin and ciprofloxacin are bactericidal agents at very low concentrations for broad spectrum of gram negative and gram positive bacteria as well as mycoplasma [11].

An attempt is made in the present study to investigate the interaction of diclofenac with enrofloxacin whether the use of diclofenac in conjunction with enrofloxacin may be advised or not.

Objectives of the present investigation were (i) to determine the concentrations of diclofenac at different time intervals in plasma and urine when it was given alone or in combination with enrofloxacin following i.v. administration, (ii) to determine the kinetic parameters of diclofenac between both groups, (iii) to compare the differences in concentrations of diclofenac in plasma and urine, and various kinetic parameters between both groups.

Materials and Methods

Experimental animals and drugs

The study was conducted on five clinically healthy female buffalo calves of unidentified breed between 12 to 18 months of age and 102 to 175 kg body weight. The buffalo calves were housed in the animal shed with concrete floor. The animals were maintained on dry fodder, cattle feed along with routine grazing and water *ad lib.*

Diclofenac and enrofloxacin were used in the present experiment. Zobid®, an injectable commercial preparation containing diclofenac sodium in concentration of 25 mg/ml marketed by Ambalal Sarabhai Enterprises Ltd., India and Enrocin® (10%), an injectable commercial preparation containing enrofloxacin in concentration of 100 mg/ml marketed by Ranbaxy Laboratories Ltd., India were used in the present experiment. Diclofenac (1 mg/kg, i.v.) was given in each of five buffalo calves and in the same animals the drug was

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again given in the same dose rate along with enrofloxacin (4 mg/kg, i.v.) in two different syringes one after another immediately after an interval of three weeks.

Collection and storage of blood and urine samples

Diclofenac (1 mg/kg) was injeted into the jugular vein in each buffalo calf and the samples of blood were collected from the contralateral jugular vein into heparinised glass centrifuge tubes before and at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 30, 36 and 48 h post administration. Similarly diclofenac (1 mg/kg) was given simultaneously with enrofloxacin (4 mg/kg) into the jugular vein and the samples of blood were collected similarly in the same animals as noted above. Simultaneously samples of urine were also collected at the above noted times. For collection of urine, a Foley's balloon catheter was introduced into the bladder through the urethra and kept in position by inflating the balloon by giving 25 to 30 ml of water. The plasma was separated after centrifugation at 3000 r.p.m. for 10-15 min at room temperature and kept in a refrigerator until analyzed, usually within three days of collection. Plasma and urine collected prior to administration of the drug were used for preparing drug standards in the respective biological fluid.

Method of analysis

Concentrations of diclofenac in plasma and urine were estimated by the reverse phase partition chromatography by using High Performance Liquid Chromatography (HPLC). The determination of diclofenac in respective biological fluid was done by the HPLC method as described [7] with slight modifications. The limit of quantitation of the method is 0.01 g/ml.

Calculation of kinetic parameters

Log plasma drug concentration versus time profile showed a biphasic curve and thus followed a 2-compartment open model as described [2, 9, 13]. Various kinetic parameters were obtained by least square regression method [10, 13]. The drug concentrations in plasma can be expressed by the following bi-exponential mathematical expression as a function of time:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where, C_p = concentration of the drug in plasma, α and β = distribution and elimination rate constants, respectively, A and B = zero time intercept of distribution and elimination phases, respectively, e = base of natural logarithm and t = time elapsed after drug administration.

Elimination rate constant (β) and zero time concentration during elimination phase (B) were obtained by the least square regression method from the terminal slope of the biphasic curve. The theoretical plasma concentration for the elimination phase can be calculated during distribution phase of various time intervals. Subtracting the theoretical value from the observed values during distribution phase, a series of residual concentrations were obtained. From these residual concentrations, distribution rate constant (α) and zero time concentration during distribution phase (A) were calculated as per the method adopted for calculation of β and B.

Statistical analysis

Concentrations of diclofenac in plasma and urine at various time intervals and its kinetic parameters when diclofenac was given alone or in combination with enrofloxacin in buffalo calves were compared by using paired t-test [17].

Results

Comparison of drug concentrations in plasma and urine

Fig. 1 depicts the comparison of plasma and urine concentrations of diclofenac when given alone or in combination with enrofloxacin in buffalo calves at various time intervals following i.v. administration. Significantly lower ($p<0.05$) levels of plasma concentration were obtained in combined administration of diclofenac with enrofloxacin (0.042 to 3 h), whereas significantly higher ($p<0.05$) levels of plasma drug concentrations were obtained at later period (8 to 24 h). Significantly lower levels ($p<0.05$) of urine drug concentrations were obtained from 0.167 to 1.5 h, whereas significantly higher levels ($p<0.01$) of urine drug concentrations were observed at later period (4 to 48 h) when diclofenac was given in combination with enrofloxacin. The peak urine drug concentration was attained earlier at 0.25 h when diclofenac was given alone and at 4 h when it was given together with enrofloxacin.

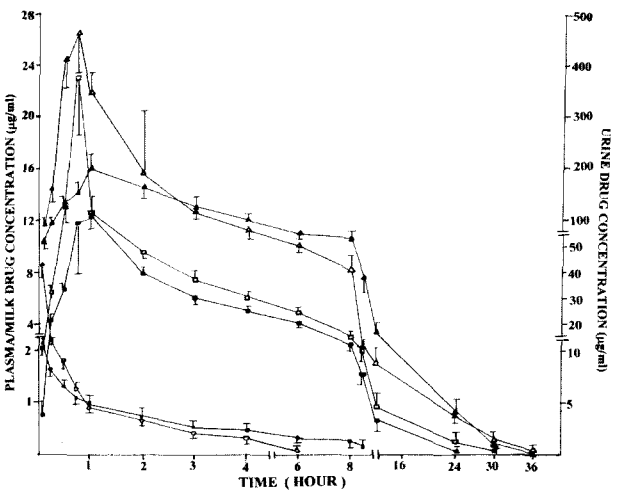

Fig. 1. Showing comparison of concentrations(㎍/㎖) of ciprofloxacin in biological fluids of afebrile and febrile goats (n=6) following single i.v. administration(4 ㎎/㎏). (●) plasma conc. in afebrile goats; (○) plasma conc. in febrile goats; (□) milk conc. in afebrile goats; (■) milk conc. in febrile goats; (△) urine conc. in afebrile goats; (▲) urine conc. in febrile goats.

Comparison of kinetic parameters of diclofenac

Statistical comparison of various kinetic parameters of diclofenac when given alone or in combination with enrofloxacin in buffalo calves is shown in Table 1. The values observed for the extrapolated zero time concentration during distribution phase (A), theoretical zero time concentration (C_p^0) and elimination rate constant (β) were significantly lower ($p<0.05$) whereas, the values of elimination half life ($t_{1/2,\beta}$), area under first moment curve (AUMC), mean residential time (MRT) and various values of volume of distribution (Vd_c, Vd_b, Vd_{area} and Vd_{ss}) were significantly higher ($p<0.05$) in combined administration of diclofenac with enrofloxacin as compared to when it was given alone. All other kinetic parameters are almost similar in both groups.

Discussion

The calculated value of distribution half life for diclofenac was found to be lower in animals when the drug was given in conjunction with enrofloxacin though it was found to differ nonsignificantly as compared to when it was given alone. This denotes that there is no kinetic interaction between diclofenac and enrofloxacin with respect to the distribution of diclofenac. Distribution half-life of 0.34 ± 0.08 h was obtained for diclofenac when given alone which denotes faster distribution of this drug in the body of buffalo calves.

The calculated values of diclofenac for the elimination half life ($t_{1/2\beta}$) obtained for both groups in the present investigation (Table 1) are very high as compared to 1.1 h after i.v. administration [19] and 1.15 h after i.m. injection [12] of diclofenac in man which denote that the drug is expected to be removed at a slower rate from the body of buffalo calves as compared to man. But, significantly ($p< 0.01$) higher $t_{1/2,\beta}$ was obtained in combined administration of diclofenac with enrofloxacin as compared to when diclofenac was given alone. This indicates that there is definite kinetic interaction and that diclofenac is expected to be removed at a slower rate in buffalo calves when combined with enrofloxacin. This fact is further supported by lower value of rate constant of drug elimination from central compartment (k_{el}) obtained in combined administration of diclofenac with enrofloxacin (0.14 ± 0.01 h^{-1}) as compared to the value of 0.64 ± 0.12 h^{-1} when diclofenac was given alone.

Significantly higher values of AUMC and MRT in combined administration of diclofenac with enrofloxacin in the present investigation reflect that the drug remains in the body of buffalo calves for a comparatively longer duration in combined administration.

The reported values for the volume of distribution of 0.17 ± 0.11 L/kg in man [19] is significantly lower to that of 0.54 ± 0.10 L/kg observed when diclofenac was given alone in the present study. The Vd_{area} obtained in combined administration of diclofenac with enrofloxacin in this investigation (1.34 ± 0.04 L/kg) is significantly higher than that of diclofenac given alone ($p<0.01$). This indicates that there may be extensive penetration of diclofenac in various body fluids and inflammatory tissues during combined administration of diclofenac with enrofloxacin which may be beneficial to the animals since the drug may be expected to reach the sites of action (inflammed tissues, synovial fluid, etc).

A large volume of distribution (> 1 L/kg) denotes wide distribution of drug throughout the body or extensive tissue binding or rapid elimination or combination of all the above [2]. In the present study, highly significant ($p<0.01$) increase in urinary excretion of diclofenac was noted for a longer duration (4 to 48 h) when it was given in conjunction with enrofloxacin as compared to its single administration. Apart from the suggested extensive penetration of diclofenac in body fluids and tissues, the significant increase in urinary excretion of diclofenac when combined with enrofloxacin might have contributed to the significant increase in Vd values as compared to its single administration. Pharmacodynamic interaction of ciprofloxacin with diclofenac in causing epileptogenic effect in mice was demonstrated [16].

Such types of studies may need to be conducted to know the dynamic interactions of diclofenac with enrofloxacin in animals.

The total body clearance (Cl_B) of the drug is noted to differ only nonsignificantly between both groups (Table 1). A higher Cl_B value of 4.2 ± 0.9 ml/kg/min in man [19] was recorded. The total plasma clearance (Cl_B) in the Yucatan minipig was five fold slower than in man [14]. The above facts indicate that the drug may be eliminated at a faster rate in as compared to buffalo calves and minipigs.

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References

1. **Altreuther, P.** Data on chemistry and toxicology of Baytril. Vet. Med. Rev. 1987, **2**, 87-89.
2. **Baggot, J. D.** Principles of Drug Disposition in Domestic Animals. The Basis of Veterinary Clinical Pharmacology. 1st ed. W.B. Saunders Company. Philadelphia, 1977.
3. **Baudtiz, R.** Enrofloxacin clinical evaluation in several animal species. Veterinary Pharmacology, Toxicology and Therapy in Food Producing Animals. pp. 21-26. Budapest University of Veterinary Science, Unipharma Company Ltd., 1990.
4. **Bergan, T., Dalhoff, A. and Rohwedder, R.** Pharmacokinetics of ciprofloxacin. Infection 1988, **16**, 3-13.
5. **Brodgen, R. N., Heel, R. C., Pakes, G. E., Speight,**

Table 1. Comparison of kinetic parameters of diclofenac when given alone (1 mg/kg) and when given together with enrofloxacin (4 mg/kg) in buffalo calves following intravenous administration

Kinetic Parameter	Mean ± S.E.M. (n = 5)	
	Diclofenac given alone	Diclofenac + enrofloxacin given together
Zero time concentration (μg/ml)		
Distribution (A)	5.74 ± 1.20	1.21 ± 0.22*
Elimination (B)	1.65 ± 0.35	0.73 ± 0.03+
C_p^0 (A + B)	7.38 ± 1.49	1.94 ± 0.22*
Rate constant (h^{-1})		
Distribution (α)	2.76 ± 0.81	4.47 ± 1.09+
Elimination (β)	0.19 ± 0.03	0.06 ± 0.01**
Halflife (h)		
Distribution ($t_{1/2,\ \alpha}$)	0.34 ± 0.08	0.21 ± 0.06+
Elimination ($t_{1/2,\ \beta}$)	4.06 ± 0.59	12.84 ± 1.29**
Microrate constant for drug transfer (h^{-1})		
Central to peripheral compartment (k_{12})	1.48 ± 0.53	2.64 ± 0.79+
Peripheral to central compartment (k_{21})	0.83 ± 0.22	1.75 ± 0.41+
Elimination from central Compartment (k_{el})	0.64 ± 0.12	0.14 ± 0.01*
Area under Curve		
AUC (mg · h/L)	11.24 ± 0.48	13.99 ± 1.59+
Area under first moment curve		
AUMC (mg · h^2/L)	51.78 ± 7.30	264.8 ± 58.10*
Mean residential time		
MRT (h)	4.72 ±0.85	18.07 ± 1.92*
Volume of distribution (L/kg)		
Vd_C	0.17 ± 0.05	0.54 ± 0.05*
Vd_B	0.72 ± 0.13	1.38 ± 0.06*
Vd_{area}	0.54 ± 0.10	1.34 ± 0.04**
Vd_{ss}	0.43 ± 0.10	1.31 ± 0.03**
Total body clearance		
Cl_B (ml/kg/min)	1.52 ± 0.07	1.24 ± 0.15+

+Non significant, *$p<0.05$, **$p<0.01$.

T.M. and Avery, G.S. Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. Drugs 1980, **20**, 24-28.

6. **Chu, D. T. W. and Fernandes, P. B.** Structure-activity relationships of the fluoroquinolones. Antimicrob. Agents Chemother. 1989, **33**, 131-135.
7. **El-Sayed, Y. M., Abdel-Hameed, M. E., Suleiman, M. S. and Najib, N. M.** A rapid and sensitive high performance liquid chromatography method for the determination of diclofenac sodium in serum and its use in pharmacokinetic studies. J. Pharm. Pharmacol. 1988, **40**, 727-729.
8. **Flammer, K., Aucoin, D. P. and Whitt, D. A.** Intramuscular and oral disposition of enrofloxacin in African grey parrots following single and multiple doses. J. Vet. Pharmacol. Ther. 1991, **14**, 359-366.
9. **Gibaldi, M. and Perrier, D.** Pharmacokinetics. Marcell Dekker Inc., New York, 1975.
10. **Gibaldi, M. and Perrier, D.** Pharmacokinetics, pp. 443-444. Marcell Dekker Inc., New York, 1982.
11. **Hooper, D. C. and Wolfson, J. S.** Fluoroquinolone antimicrobial agents. New England J. Med. 1991, **7**, 384-394.
12. **Kurowski, M.** Pharmacokinetics and biological availability of diclofenac preparations following intramuscular injection of 75 mg and oral administration of 150 mg of active drug. J. Rheumatol. 1988, **1**, 37-42.
13. **Notari, R. E.** Biopharmaceutics and Clinical Pharmacokinetics. 3rd ed. Marcell Dekker Inc., New York, 1980.
14. **Oberle, R. L., Das, H., Wong, S. L., Chan, K. K. and Sawchuk, R. J.** Pharmacokinetics and metabolism of diclofenac sodium in Yucatan miniature pigs. Pharm. Res. 1994, **11**, 698-703.
15. **Scheer, M.** Studies on the antimicrobial activity of Baytril. Vet. Med. Rev. 1987, **2**, 90-99.
16. **Shrivastava, M. P., Makde, S. D. and Paranjpe, B. D.** Interaction of ciprofloxacin with diclofenac and paracetamol in relation to its epileptogenic effect. Indian J. Physiol.

Pharmacol. 1997, **41**, 164-166.

17. **Snedecor, G. W. and Cochran, W. G.** (ed.). Statistical Methods. 6th ed., Oxford and IBH Publishing Company, New Delhi, India, 1967.
18. **Tyczkowska, K., Hedeen, K. M., Aucoin, D. P. and Aronson, A. L.** High performance liquid chromatography method for the simultaneous determination of enrofloxacin and its primary metabolite ciprofloxacin in canine serum and prostatic tissues. J. Chromato. 1989, **493**, 337-346.
19. **Willis, J. V., Kendall, M. J., Flinn, R. M., Thornhill, D. P. and Welling, P. G.** The pharmacokinetics of diclofenac sodium following intravenous and oral administration. Eur. J. Clin. Pharmacol. 1979, **16**, 405-410.