

Gatifloxacin pharmacokinetics in buffalo calves after single intramuscular administration

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ABSTRACT

The pharmacokinetics and *in vitro* plasma protein binding of gatifloxacin after a single intramuscular injection of 4 mg kg⁻¹ were studied in buffalo calves. The minimum therapeutic concentration of drug was maintained in plasma from 1 min to 12 h. Gatifloxacin was rapidly absorbed from the extravascular site of injection, as evident from the high value of absorption rate constant (4.91 ± 0.22 h⁻¹) and attained a C_{max} of 2.98 ± 0.08 µg mL⁻¹ at 1h. The area under the plasma concentration-time curve and apparent volume of distribution were 10.8 ± 0.64 µg mL⁻¹ h and 3.2 ± 0.08 L kg⁻¹, respectively. Elimination half-life and total body clearance were 7.45 ± 0.55 h and 301.5 ± 34.4 mL kg⁻¹h⁻¹, respectively. C_{max}/MIC ratio was 14.9 ± 0.3 and systemic bioavailability was 79.7 ± 3.35 per cent. Gatifloxacin was bound to plasma proteins of buffalo calves to the extent of 25.0 ± 1.05 per cent. A suitable intramuscular dosage regimen of gatifloxacin in buffalo calves would be 6.0 mg kg⁻¹ followed by 5.3 mg kg⁻¹ at 24 h intervals.

Key words: buffalo calves, dosage, gatifloxacin, pharmacokinetics

Introduction

Gatifloxacin, a recently introduced fluoroquinolone, possesses good activity against a wide range of gram-positive and gram-negative pathogens, atypical organisms and some anaerobes (PERRY et al., 2002). It is commonly indicated for the treatment of acute bacterial sinusitis, chronic bronchitis, pneumonia, urinary tract infections, acute pyelonephritis and gonorrhoea (DANIEL, 2001). Fluoroquinolone resistance relates directly to human and veterinary usage and emerging bacterial resistance poses the single greatest threat to

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the future survival of fluoroquinolone drugs as an antibiotic class (BAKKEN, 2004). As a member of respiratory quinolones, gatifloxacin possesses enhanced activity against *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* (FISH and NORTH, 2001). If a drug is to be used effectively, it is important to investigate its pharmacokinetics in each animal species and the climate in which the drug is to be used clinically (NAWAZ et al., 1980). Accordingly, pharmacokinetic studies of gatifloxacin have been conducted in rabbits (LUTSAR et al., 1998), mice (ANDES and CRAIG, 2002) and humans (NAKASHIMA et al., 1995). However, there is no information available on the pharmacokinetic data of gatifloxacin in buffalo species. Keeping in view the marked species variation in the kinetic data of antimicrobial drugs, the present study was planned to determine the pharmacokinetics, *in vitro* plasma protein binding and appropriate dosage regimen of gatifloxacin in buffalo calves (*Bubalus bubalis*) after its single intramuscular administration.

Materials and methods

The study was conducted on 6 healthy male buffalo calves of non-descript breed weighing 90-130 kg and about one year of age. The animals were maintained in the departmental animal shed on seasonal green fodder and water *ad libitum*. Average daytime temperature was about 25 °C during the experimental period. Gatifloxacin (Gatiquin, Cipla Ltd., Ahmedabad, India) was administered intramuscularly at the dose rate of 4 mg kg⁻¹ body mass.

Blood samples (5 mL) were withdrawn from the jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 10, 15 and 30 min. and at 1, 2, 4, 6, 8, 10, 12, 16 and 24 h after administration of drug. Plasma was separated by centrifugation at 2000 g for 15 min. at room temperature and kept at -20 °C until analysis, which usually took place on the day after collection.

Concentration of gatifloxacin in plasma samples was estimated by a standard microbiological assay technique (ARRET et al., 1971) using *Escherichia coli* (ATCC 10536) as the test organism. The test organism was cultured on antibiotic medium no. 1 at 37 °C for 24 h and a suspension was prepared in sterile normal saline. 20 mL of molten seed layer containing bacterial suspension was poured on a Petri dish with the help of Cornwell Continuous Pipetting Device (Becton Dickinson, New Jersey, USA). Preliminary experiments were conducted to determine the actual amount of bacterial suspension to be used in the preparation of seed layer. After solidification of the media, six wells were punched at equal distances with using a punching device (developed and standardized in our laboratory).

Three alternate wells were filled with one plasma sample and the remaining three wells with a standard reference solution of gatifloxacin (0.2 µg mL⁻¹). These assay plates were incubated at 34 °C for a period of 12 h. At the end of incubation period the diameters of

zone of inhibition of each well was measured with a Fisher Lilly Antibiotic Zone Reader (Fisher Scientific Company, New Jersey, USA). Nine replicates were analysed for each sample. The repeatability of this method was excellent and within-day error estimation was less than 2%. The minimum detection level was $0.05 \mu\text{g mL}^{-1}$ of gatifloxacin.

Based on diameter of zone of inhibition of the reference concentrations, the plasma levels of gatifloxacin were calculated and expressed as $\mu\text{g mL}^{-1}$. Pharmacokinetic parameters were calculated manually by the least squares regression technique (GIBALDI and PERRIER, 1982).

The *in vitro* plasma protein binding was determined by the equilibrium dialysis technique (KUNIN et al., 1959) at plasma concentrations of 0.1, 0.5, 1, 5, 10 and $20 \mu\text{g mL}^{-1}$ of gatifloxacin and the constants for protein binding were obtained (PILLOUD, 1973).

Results

Table 1. Pharmacokinetic parameters of gatifloxacin in buffalo calves (n = 6) following single intramuscular dose of 4 mg kg^{-1} body mass

Parameter	Unit	Mean \pm SE
A'	$\mu\text{g mL}^{-1}$	1.43 ± 0.10
Ka	h^{-1}	4.91 ± 0.22
t_{Ka}	h	0.13 ± 0.01
B	$\mu\text{g mL}^{-1}$	1.04 ± 0.03
β	h^{-1}	0.09 ± 0.008
t_{β}	h	7.45 ± 0.55
AUC	$\mu\text{g mL}^{-1} \cdot \text{h}$	10.8 ± 0.64
AUMC	$\mu\text{g mL}^{-1} \cdot \text{h}_c$	122.9 ± 15.2
Vd(area)	L kg^{-1}	3.20 ± 0.08
Cl_b	$\text{mL kg}^{-1} \text{ h}^{-1}$	301.5 ± 34.4
MRT	h	11.0 ± 0.80
td	h	32.2 ± 2.4
C_{max}	$\mu\text{g mL}^{-1}$	2.98 ± 0.08
t_{max}	h	1.0 ± 0.00
AUC/MIC	ratio	54.3 ± 3.2
Cmax/MIC	ratio	14.9 ± 0.30

The mean value for bioavailability (F) was $79.7 \pm 3.35\%$. A' and B = zero-time plasma drug concentration intercepts of the regression lines of absorption and elimination phases, respectively;

K_a and β = absorption and elimination rate constants, respectively; $t_{1/2Ka}$ = absorption half-life; $t_{1/2\beta}$ = elimination half-life; AUC = area under the plasma concentration-time curve; AUMC = area under the first moment curve; $Vd(\text{area})$ = apparent volume of distribution; Cl_B = total body clearance; MRT = mean residence time; td = duration of therapeutic effect; C_{max} and t_{max} = peak plasma drug concentration and time required to attain peak concentration, respectively; MIC = minimum inhibitory concentration of drug in plasma.

Table 2. *In vitro* binding of gatifloxacin to plasma proteins of buffalo calves.

Parameter	Unit	Mean \pm SE
β_i	Mol g^{-1}	4.867×10^{-8}
K_β	Mol	5.39×10^{-6}
Extent	%	25.0 ± 1.05

Each value is the mean of results obtained from three experiments at different plasma concentrations ranging from 0.1 to 20 $\mu g mL^{-1}$. β_i = binding capacity of plasma -proteins with gatifloxacin. K_β = dissociation rate constant of the protein-drug complex.

Mean plasma concentrations of gatifloxacin as a function of time were plotted on a semilogarithmic scale (Fig. 1). Following intramuscular administration a mean plasma drug concentration of $0.22 \pm 0.008 \mu g mL^{-1}$ was detected at 1 min. Peak drug concentration of

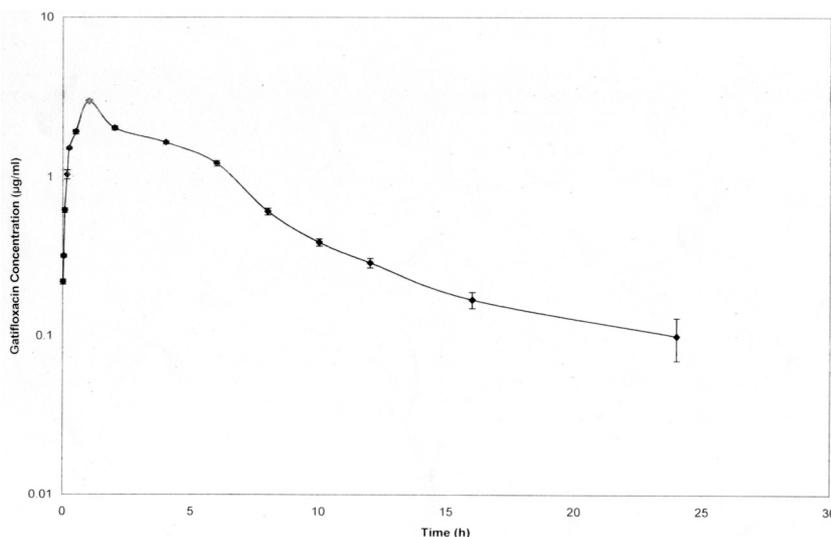


Fig. 1. Semilogarithmic plot of plasma concentration-time profile of gatifloxacin following its single intramuscular injection of 4 mg kg^{-1} in buffalo calves. Values are presented as mean \pm SE of the results obtained from 6 animals.

$2.98 \pm 0.07 \mu\text{g mL}^{-1}$ was attained 1h. after injection and was followed by a gradual decline to $0.29 \pm 0.02 \mu\text{g mL}^{-1}$ at 12 h.

Evaluation of results on observed plasma levels revealed that the disposition of gatifloxacin was best fitted to a one-compartment open model and adequately described by the exponential equation $C_p = Be^{-\beta t} - A'e^{-Ka t}$, where C_p is the drug concentration at time t , A' and B are zero-time intercepts of absorption and elimination phases, respectively. K_a and β are the absorption and elimination rate constants, respectively, and e represents the base of natural logarithm.

Based on the plasma drug levels, various pharmacokinetic parameters pertaining to the absorption and elimination of gatifloxacin in buffalo calves were calculated and are presented in Table 1. The extent and capacity of *in vitro* plasma protein binding of gatifloxacin are presented in Table 2.

Discussion

The minimum therapeutic plasma drug concentration was maintained from 1 min. to 12 h of administration. The MIC_{90} of gatifloxacin against various organisms has been reported to be 0.015 to $8 \mu\text{g mL}^{-1}$ (ANDES and CRAIG, 2002). However, in view of the influences of different factors *in vivo*, in the present discussion, a concentration of $0.2 \mu\text{g mL}^{-1}$ has been considered as the MIC of gatifloxacin. Gatifloxacin undergoes minimal hepatic metabolism and after single oral doses, up to 88% of the administered drug has been reported to be recovered unchanged in urine within 72 h (NAKASHIMA et al., 1995). Due to negligible metabolism the chances of any active metabolite being considered as parent compound in bioassay is greatly reduced. The early appearance of gatifloxacin in plasma suggested rapid absorption of the drug into systemic circulation, which was further confirmed by the high values of absorption rate constant ($4.91 \pm 0.22 \text{ h}^{-1}$). Rapid absorption following intramuscular injection has also been reported for another fluoroquinolone: enrofloxacin in buffalo bulls (VERMA et al., 1999). The high values of AUC ($10.8 \pm 0.64 \mu\text{g mL}^{-1} \cdot \text{h}$) and AUMC ($122.9 \pm 15.2 \mu\text{g mL}^{-1} \cdot \text{h}^2$) of gatifloxacin in buffalo calves indicated that a vast area of body fluids and tissues is covered by drug concentration after intramuscular injection. High value of AUC has also been reported for gatifloxacin ($32.4 \text{ mg} \cdot \text{h} \cdot \text{L}^{-1}$) after oral administration in man (NAKASHIMA et al., 1995) and for the other fluoroquinolones: marbofloxacin ($15.3 \mu\text{g mL}^{-1} \cdot \text{h}$) and danofloxacin ($10.11 \mu\text{g mL}^{-1} \cdot \text{h}$) in cattle (THOMAS et al., 1994; SHOJAEI, 1997).

The elimination half-life of gatifloxacin in buffalo calves ($7.45 \pm 0.55 \text{ h}$) was in agreement with the findings in human beings, where the half-life of gatifloxacin after oral administration was 7-8 h (MIGNOT et al., 2002; NAKASHIMA et al., 1995). However, a low value of $t_{1/2\beta}$ ($0.6-1.0 \text{ h}$) has been reported for gatifloxacin in mice (ANDES and CRAIG, 2002). A short elimination half-life has also been reported for other fluoroquinolones:

enrofloxacin (1.97 h) in buffalo bulls (VERMA et al., 1999) and danofloxacin (4.33 h) in cattle (SHOJAEI, 1997). In contrast, ciprofloxacin was found to have a long elimination half-life of 10.3 h after intramuscular administration in cattle (SINGH and SRIVASTAVA, 2000). Total body clearance (Cl_B) of gatifloxacin in buffalo calves was 301.5 ± 34.4 mL $kg^{-1} h^{-1}$. Comparable values of Cl_B have been reported for gatifloxacin ($188-209$ mL min^{-1}) after oral dose in humans (NAKASHIMA et al., 1995) and enrofloxacin (210.2 mL $kg^{-1}h^{-1}$) after intramuscular dose in buffalo bulls (VERMA et al., 1999).

C_{max}/MIC ratio has been predicted as an effective index to determine the relationship between *in vivo* drug exposure and extent of bacterial killing. A ratio of 8 has been suggested as the optimum C_{max}/MIC against *S. pneumoniae* for gatifloxacin (FISH and NORTH, 2001). The C_{max}/MIC ratio of gatifloxacin was 14.9 ± 0.3 following its intramuscular injection in buffalo calves, suggesting much higher plasma levels to produce desired bactericidal effects. The systemic bioavailability, which indicates the percentage of drug absorbed from the extravascular site of injection to the central compartment, was 79.7 ± 3.35 per cent after intramuscular administration of gatifloxacin in buffalo calves. In support of the present findings, a high value of bioavailability was reported after an oral dose of gatifloxacin (96%) in human (LACRETA et al., 1999) and following intramuscular injection of norfloxacin (73%) in cattle (GIPS and SOBACK, 1996).

Gatifloxacin was bound to the plasma proteins of buffalo calves to the extent of 25.0 ± 1.05 per cent. In confirmation to the present findings, 20% serum binding of gatifloxacin has been reported in human beings (NAKASHIMA et al., 1995). Further, the majority of the fluoroquinolones have been shown to exhibit low plasma protein binding, predominantly to albumin, ranging from 20-40 per cent (BERGOGNE-BEREZIN, 2002). However, from the low value of β_1 it may be inferred that the binding of gatifloxacin to plasma proteins is weak in buffalo calves and that it is reversible, as evident from the high value of the dissociation rate constant of the protein drug complex.

Taking a convenient dosage interval of 24 h, the priming and maintenance doses of gatifloxacin, to maintain the minimum a therapeutic plasma level of $0.2 \mu g mL^{-1}$, were calculated to be 5.97 and 5.33 mg kg^{-1} , respectively. However, under field conditions a suitable intramuscular dosage regimen of gatifloxacin in buffalo calves would be 6.0 mg kg^{-1} followed by 5.3 mg kg^{-1} at 24 h intervals.

The high values of AUC, Vd(area), $t_{1/2\beta}$ and C_{max}/MIC ratio and moderate bioavailability, along with rapid absorption following intramuscular administration of gatifloxacin, suggested that gatifloxacin may be effectively employed by intramuscular injection in the treatment of bacterial infections in buffalo calves.

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SAŽETAK

Farmakokinetika i *in vivo* vezanje gatifloksacina na proteine plazme istraživani su u bivolje teladi nakon jednokratne intramuskularne primjene u dozi od 4 mg/kg. Minimalna terapijska koncentracija lijeka održavana je u plazmi u tijeku od 1 minute do 12 sati. Gatifloksacin se brzo resorbirao s mjesta ubrizgavanja što je vidljivo po visokoj vrijednosti konstante brzine apsorpcije ($4,91 \pm 0,22$ sati) i postignute C_{\max} $2,98 \pm 0,08$ $\mu\text{g/mL/sat}$. Površina ispod koncentracijske krivulje u plazmi iznosila je $10,8 \pm 0,64$ $\mu\text{g/mL/sat}$, a prividni volumen raspodjele $3,2 \pm 0,08$ L/kg. Poluvrijeme izlučivanja iznosilo je $7,45 \pm 0,55$ sati, a ukupni tjelesni klirens $301,5 \pm 34,4$ mL/kg/sat. Omjer C_{\max}/MIC bio je $14,9 \pm 0,3$, a sustavna bioraspoloživost iznosila je $79,7 \pm 3,35\%$. Gatifloksacin je bio vezan na bjelančevine plazme do $25,0 \pm 1,05\%$. Prikladno intramuskularno doziranje u bivolje teladi bilo bi 6,0 mg/kg, a u sljedećim dozama treba davati 5,3 mg/kg u razmacima od 24 sata.

Ključne riječi: bivolja telad, doziranje, gatifloksacin, farmakokinetika
