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Modification of the pharmacokinetics and dosage of danofloxacin by endotoxin-induced fever in buffalo calves

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Danofloxacin is a fluoroquinolone antimicrobial drug with rapid bactericidal activity against a broad range of pathogens responsible of respiratatory tract infections, urinary tract infections, gonorrhoea and a number of disease syndromes of economic importance in the commercial rearing of livestock (Norcia 1999, Daniel 2001). The pharmacokinetics of danofloxacin has been investigated in cow (Shem-Tov *et al.* 1998), calves (McKellar *et al.* 1999), sheep (Mckellar *et al.* 1998, Shem-Tov *et al.* 1997), goats (Aliabadi *et al.* 2001, Atef *et al.* 2001), camel (Aliabadi *et al. 2*003), horses (Fernández *et al.* 2006) and rabbits (Fernández *et al.* 2007). However, such information is completely lacking in buffaloes. Disease conditions are known to alter markedly the pharmacokinetics of antimicrobials (Nakamura *et al.* 1989). Fever is the most important manifestation of many infectious diseases and is reported to induce biochemical and physiological alterations in cells (Van Miert 1987, Lohuis *et al.* 1988). Hence the present study was undertaken to determine the pharmacokinetics and urinary excretion and dosage regimen of danofloxacin in healthy and febrile buffalo calves.

Male buffalo calves (4), with weights ranging from 65–110 kg, were housed in an animal shed with a concrete floor and adequate ventilation. A constant supply of water was maintained in the shed. All the animals were acclimatized in the animal shed under uniform conditions and were maintained on green fodder of the season and wheat straw. The animals were apparently healthy at the time of experiment. Danofloxacin myselate was injected at 1.25 mg/kg body weight into the jugular vein and blood samples were collected from contralateral jugular vein of all the 4 animals at 2.5, 5, 7.5, 10, 15, 30, 45, 60 min and 2, 3, 4, 6, 8, 12, 16 and 24h after administration of the drug. Plasma was separated by centrifugation at 3000 rpm for 15 min at room temperature and stored at – 20°C. Samples from urine voided by animals were collected at 2, 4, 6, 8, 12, 18 and 24h. These animals were kept in metabolic stalls so that all the urine passed by the animals could be collected without contamination. After a wash-out period of 20 –30 days, fever was induced in the same animals by intravenous administration of 1µg/kg body weight of *E.coli* endotoxin (lipopolysaccharide). Danofloxacin was administered intravenously when there was rise in rectal temperature by 1.7°C. The dose, route of administration of drug and sampling times were kept the same as those in healthy animals.

The concentration of danofloxacin in plasma and urine was estimated by a micro-biological assay technique (Arret *et al.* 1971) using *Escherichia coli* (MTCC739) as the test organism. Standard curve of danofloxacin was prepared in phosphate buffer and plasma of healthy buffalo calves in the concentration ranging from 0.0125 to 1.0 µg/ml. However during the test assay a reference concentration (0.025 µg/ml) was run along with each sample. The assay could detect 0.0125 µg/ml of danofloxacin. The data from each animal were assayed separately to calculate the pharmacokinetic parameters. The plasma concentration – time curve of danofloxacin followed two-compartment open model in buffalo calves. The pharmacokinetic parameters were calculated by two compartment open model as described by Gibaldi and Perrier (1982). The difference between two means based on the individual observations was determined by Student's t test. The significance was assessed at $P< 0.05$ (Singh *et al.* 1991). The plasma concentration of danofloxacin after intravenous administration in healthy and endotoxin induced febrile buffalo calves are drawn on a semi logarithmic scale.

Evaluation of the data revealed that the disposition kinetics of danofloxacin was best described by a two compartment open model. The plasma concentration time data were adequately described by the equation:

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

where, Cp is the danofloxacin concentration at time't'. A and B are zero time intercepts of the distribution and

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elimination phases, respectively, of plasma concentration time curve; α and β are the distribution and elimination rate constants, respectively, and 'e' represents the base of natural logarithm. Danofloxacin has also been reported to follow two compartment open model in horses (Fernández *et al.* 2006), rabbits (Fernández *et al.* 2007), cattle (Giles *et al.* 1991), goats (Aliabadi and Lees 2001, Atef *et al.* 2001), calves (Apley and Upson 1993) and camel (Aliabadi *et al.* 2003). In healthy buffalo calves the highest plasma level of danofloxacin was 0.80±0.021 µg/ml at 2.5 min. The levels rapidly declined to 0.25±0.023 µg/ml at 1h and thereafter the danofloxacin plasma levels slowly declined and were detected up to 24h. In febrile buffalo calves, the peak plasma concentration of danofloxacin was 0.72±0.032 µg/ml at 2.5 min which rapidly declined to 0.20±0.006 µg/ml at 1h; thereafter, the levels gradually decreased to 0.01±0.001 µg/ml at 24h. There was no significant difference in the danofloxacin plasma concentrations of healthy and febrile animals.

The initial plasma concentration of danofloxacin in febrile calves (0.72±0.032) was lower than that in healthy animals (0.80±0.021 µg/ml), similarly lower peak blood levels of antimicrobials has been reported in febrile animals, gentamicin in goats (Ahmed *et al.* 1994), cefazolin in goats (Roy *et al.* 1992), cefuroxime (Chaudhary *et al.* 1999) and ceftriaxone (Dardi *et al.* 2005) in buffalo calves.

The values of the pharmacokinetic parameters for danofloxacin in healthy and febrile buffalo calves following its intravenous administration are presented in Table 1. In healthy buffalo calves the value of distribution half-life was 0.13±0.01h and elimination half-life was 4.41±0.04h. The ratio of rate constant for the transfer of drug from central to peripheral compartment and vice versa was 3.37±0.03. The total body clearance, area under curve, apparent volume of distribution was 0.67±0.05 L/kg/h, 1.52±0.12 µg/ml×h and 4.23±0.38 L/kg, respectively, whereas in febrile buffalo calves the value of distribution half-life was 0.24±0.09h and elimination half-life was 4.83±0.11h. The total body clearance, area under curve, apparent volume of distribution were 0.71±0.03 L/kg/h, 1.40±0.06 µg/ml×h and 4.96±0.26 L/kg, respectively. Similar values for the distribution half-life ($t_{1/2}\alpha$), area under plasma concentration curve (AUC), apparent volume of distribution (Vd) and tissue/plasma concentration drug ratio (T/P) in healthy and febrile animals indicate that fever did not affect the distribution of danofloxacin in buffalo calves. Slightly elevated values of elimination half life ($t_{1/2ß}$) and mean residence time (MRT) in febrile animals indicate that the drug was excreted relatively slowly from these animals compared to the healthy subjects. However there was no statistically significant difference between the pharmacokinetic parameters of healthy and febrile animals (P >0.05). Similar results were reported with administration of cefuroxime in buffalo calves (Chaudhary *et al.* 1999) and marbofloxacin in goats (Waxman *et al.* 2003). The recovery of danofloxacin in urine of healthy and febrile animals was 5.0 and10.0%, respectively (Table 2), similarly low excretion has been reported for enrofloxacin, another fluoroquinolones in healthy (6.3%) and febrile calves (10.3%) (Ahanger and Srivastava 2000).

Table 1. Pharmacokinetics of danofloxacin in healthy and febrile buffalo calves following its single intravenous administration @1.25 mg/kg body weight (n= 4)

Parameter[a]	Unit	Healthy	Febrile
C_p^o	mg/ml	0.96±0.02	0.86±0.04
A	mg/ml	0.78±0.02	0.72±0.03
B	mg/ml	0.17±0.01	0.14±0.01
α	/h	5.10±0.24	4.59±0.28
$t_{½}\alpha$	h	0.13±0.01	0.24±0.09
β	/h	0.157±0.003	0.14±0.007
$t_{1/2}\beta$	h	4.41±0.04	4.83±0.11
K_{12}	/h	3.57±0.15	3.26±0.24
K_{21}	/h	1.05±0.05	0.86±0.04
AUC	µg/ml× h	1.52±0.12	1.40±0.06
$Vd_{(area)}$	L/kg	4.23±0.38	4.96±0.26
Cl_B	L/kg/h	0.67±0.05	0.71±0.03
T/P	Ratio	3.06±0.26	3.30±0.22
MRT	h	5.07±0.30	5.97±0.09
$V_{d\ (SS)}$	L/kg	4.56±0.11	5.52±0.39

[a]Kinetic parameters are as described by Gibaldi and Perrier (1982); values given are mean±SE of the result obtained from 4 animals. C_p^o, Plasma drug concentration immediately following intravenous injection of single dose; A and B, Zero – time plasma drug concentration intercepts of regression lines of distribution and elimination phases respectively; α and β, distribution and elimination rate constants respectively ; $t_{½}\alpha$, distribution half-life;, $t_{1/2}\beta$, elimination half-life ; AUC, area under plasma concentration time curve ; $Vd_{(area)}$ and $V_{d\ (SS)}$, apparent volumes of distribution calculated by area method and steady state levels, respectively; Cl_B, total body clearance; MRT, mean residence time; K_{12} and K_{21}, micro rate constants; T/P, tissue/plasma concentration drug ratio.

The main goal of the present study was to compute the most appropriate dosage regimen for danofloxacin. During antimicrobial therapy, the plasma concentration should not fall below the minimum inhibitory concentration (MIC). Concentrations in the range of 0.016– 0.125µg/ml are considered as the MIC for danofloxacin (Giles 1996).The maintenance dose (D') may be calculated from the equation

$$D' = Cp\ (min)^{\alpha}.\ Vd\ (e^{\beta\tau}–1)$$

where, Cp (min) $^{\alpha}$ is the minimum effective concentration of the drug, Vd (area) is the volume of distribution and β and τ are the elimination rate constant and dosage interval, respectively. The priming dose may be obtained by omitting the –1 from this equation. Taking 12h as a convenient dosage interval (τ) and MIC of 0.05µg/ml and the values of β and Vd (area) of the individual animals, the priming and maintenance doses of danofloxacin were calculated to be 1.4 mg/kg followed by 1.2 mg/kg in healthy, as well as the febrile buffalo calves.

Table 2. Comparative data of urinary excretion of danofloxacin in healthy and febrile buffalo calves following a single intravenous administration @1.25 mg/kg body weight

Time interval (hr)	Cumulative per cent of total dose excreted	
	Healthy	Febrile
0 – 2	1.57±0.13[a]	4.28±0.98*
2 – 4	2.38±0.20[a]	6.45±0.23[a]**
4 – 6	2.59±0.46	7.51±0.33[a]**
6 – 8	3.34[c]	7.80±0.55
8 – 12	3.46[c]	8.50±0.21[b]
12 – 18	3.74±0.39	8.77±0.54[b]**
18 – 24	4.53±0.36[a]	9.10±0.61[a]**

Values given at different time intervals are mean±SE of the results obtained from 4 animals unless otherwise stated.

[a] Value of 3 animals; [b] Value of 2 animals; [c] Value of single animal; * Significantly ($P < 0.05$) different as compared to corresponding values of healthy animals; ** significantly ($P < 0.01$) different as compared to corresponding values of healthy animals.

SUMMARY

The pharmacokinetics, urinary excretion and dosage regimen of danofloxacin was investigated in healthy and febrile buffalo calves (n=4) following a single intravenous administration (1.25 mg/kg). The distribution and elimination half-lives of danofloxacin were 0.13±0.01h and 4.41±0.04h, respectively, in healthy and 0.24±0.09h and 4.83±0.11h, respectively, in febrile buffalo calves. About 5.0% and 10% of administered dose was excreted in the urine of healthy and febrile animals, respectively, within 24h. Pharmacokinetics and dosage regimen of danofloxacin was not significantly altered by experimentally induced fever in buffalo calves.

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