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Effect of Ketoprofen Co-Administration and Febrile State on Pharmacokinetics of Levofloxacin in Goats Following Intravenous Administration

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A B S T R A C T

The pharmacokinetic of levofloxacin (4 mg/kg) was studied following intravenous administration of levofloxacin in ketoprofen (3 mg/kg) treated and febrile (Lipopolysaccharide induced) goats. The concentration of levofloxacin in plasma was detected by using High Performance Liquid Chromatography. No significant changes were reported in pharmacokinetic parameters following co-administration of levofloxacin with ketoprofen. While under febrile state, significant increase in area under plasma curve and decrease in total body clearance were observed. Integrating the pooled pharmacokinetic data generated from the present study, levofloxacin via intravenous administration (4 mg/kg) repeated at 12 h interval is sufficient to maintain plasma concentration above the 0.05 µg/mL MIC for most of the gram-positive and gram-negative microorganisms.

Introduction

Antibacterial and NSAIDs are used most frequently in multiple drug prescriptions. It is well documented that concurrently administered drugs may alter pharmacokinetics of one or both drugs. Levofloxacin is a third-generation fluoroquinolone with a wide spectrum of bactericidal activity. The drug is active against Gram-negative, Gram-positive and anaerobic bacteria including Pseudomonas species. It has enhanced activity against *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Enterococcus* species, besides having good activity against Mycoplasma and Chlamydia (Davis and Bryson, 1994; Martinez *et al.*, 2006). Ketoprofen is a routinely used as non-

steroidal anti-inflammatory, analgesic and antipyretic agent in veterinary practice (Boothe, 1995). Pharmacokinetics of levofloxacin, administered as single drug were investigated in many species (Albarellos *et al.*, 2005; Dumka and Srivastava, 2007[ab], Goudah, 2009; Varia *et al.*, 2009; Goudah and Hasabelnaby, 2010; Patel *et al.*, 2012[abc], Goudah and Abo-El-Sooud, 2009; Patel *et al.*, 2013). However, there is no information available on the influence of co-administration of ketoprofen and febrile state on the pharmacokinetic of levofloxacin in goats. Looking to possibility for interaction of ketoprofen and to reveal pharmacokinetic of levofloxacin in goat under febrile state, the study was undertaken to determine effect of ketoprofen and febrile condition on pharmacokinetics of levofloxacin in goat.

Materials and Methods

Experimental animals

The experiment was conducted on six healthy adult (2-3 years of age) Surti goat, weighing 28-32 kg. Each animal was housed in a separate pen and provided standard ration with *ad libitum* water. Goats were kept under constant observation for two weeks before the commencement of the experiment and subjected to clinical examination to exclude the possibility of any diseases. The experimental protocol was approved by Institutional Animal Ethics Committee.

Drug and chemical

Levofloxacin infusion (500 mg/100 mL; Tavanic®, Aventis Pharmaceutical Ltd, Bangalore) was procured from local pharmacy. Levofloxacin technical grade powder was procured from Moxi Laboratory Pvt. Ltd., Gujarat, India. Acetonitrile, Triethylamine, Perchloric acid (70%) and Ortho-phosphoric acid (min. 58%) (Analytical grade) were purchased from Merck Limited, Mumbai.

Drug administration and sample collection

All six animals were randomly allocated to receive injection of levofloxacin at the dose rate of 4 mg/kg. A washout period of 2 weeks was observed between treatments. The intraveneous injection (4 mg/kg) was administered in jugular vein, while ketoprofen was administrated via. deep intramuscular (3 mg/kg) in gluteal muscle. Blood samples (3 mL) were collected, before administration and at 5, 10, 15, 30 min and 1, 2, 4, 8, 12, 18, 24 and 36 h after concurrent intravenous and intramuscular administration of levofloxacin and ketoprofen respectively from contralateral jugular vein. Febrile state in goat was induced by injecting lipopolysaccharide (LPS) of Escherichia coli (O55:B5) at the dose rate of 0.2 µg/kg body weight intravenously (Verma and Roy, 2006). LPS was repeated at dose rate of 0.1 and 0.05 µg/kg at 12 h and 24 h, respectively to maintain the febrile state up to 36 h. Blood samples (3 mL) were collected, before administration and at 5, 10, 15, 30 min and 1, 2, 4, 8, 12, 18, 24 and 36 h after intravenous administration of levofloxacin in febrile goats. Goats were monitored for any adverse reactions during the entire study period. Blood samples were subjected to centrifugation at 3000g for 10min and plasma samples was collected and preserved -20 ^{o}C, and analyzed within 48 h for determination of levofloxacin concentration.

Analytical assay of levofloxacin and pharmacokinetic analysis

Levofloxacin concentration in plasma samples was determined by reverse-phase High Performance Liquid Chromatography (HPLC) after extraction, using a reported assay (Varia *et al.*, 2009) with minor modifications. The High Performance Liquid Chromatography

(HPLC) apparatus (Laballiance, USA) comprised of quaternary gradient delivery pump (model AIS 2000), UV detector (model 500) and C18 column (Thermo ODS: 250 x 4.6 mm ID) were used. Pharmacokinetic data integration was done by software "Clarity" (Version 2.4.0.190). Pharmacokinetic data of levofloxacin following intravenous injection in ketoprofen treated and in febrile goats were compared to intravenous injection of levofloxacin alone in goats (Patel *et al.,* 2013)

Solution of pure enrofloxacin powder (40 µl of 0.5 mg/mL concentration) was utilized as an internal standard (IS). After adding internal standard in each plasma samples (500 µL), it was deproteinized by addition of perchloric acid (50 µl) and vortexed for one minute. This was followed by centrifugation at 3000 g for 10 minutes. An aliquot of supernatant was collected in clean vial and 20 µL was injected into loop of HPLC system using 25 µL glass syringe (Hamilton Bonaduz AG, Switzerland). The mobile phase consisted of a mixture of 1% triethylamine in water and acetonitrile (85:15 v/v) adjusted to pH 3.0 with ortho-phosphoric acid. Mobile phase was filtered by 0.45 µ size filter (Ultipor N66Nylone 6,6 membrane, PALL Pharmalab filtration Pvt., Ltd., Mumbai) and degassed by ultra-sonication. Thereafter mobile phase was pumped into column at a flow rate of 1.5 mL/min at ambient temperature. The effluent was monitored at 290 nm wavelength.

Calibration curve was prepared daily for drug concentration ranging from 0.01 to 50 µg/mL. The assay was sensitive (LLOD: 0.01 µg/mL), reproducible and linearity was observed from 0.01 to 50 µg/mL (r^2 = 0.99). The lower limit of quantification of the drug with a coefficient of variation was less than 8.36% for 0.01 µg/mL concentration. The mean extraction recovery from plasma was >82.81 ± 3.83% at the spiked concentrations between 0.01 and 50 µg/mL. Precision and accuracy were determined using quality control (QC) samples at concentrations 0.05, 1, 2.5, 10 and 50 µg/mL (5 replicates each per day). The intraday and interday coefficients of variation for 5 QC samples were satisfactory with the relative deviations (RSD) of less than 9.77 %.

PK/PD integration

The peak plasma drug concentration (C_{max}) and area under the curve ($AUC_{(0-\infty)}$) were applied in the calculation of the predictors of efficacy (C_{max}/MIC and $AUC_{(0-\infty)}$/MIC) for levofloxacin following intravenous administration in ketoprofen treated and febrile goat. MIC_{90} of 0.05 µg/mL of levofloxacin has been taken into consideration to determine dosage of levofloxacin.

Statistical analysis

Levofloxacin plasma concentration and pharmacokinetic parameters of different treatment groups were compared by students' "t" test using SPSS software (version 12.0.1).

Results and Discussion

Plasma levofloxacin concentrations at different time intervals following intravenous injection under febrile state and ketoprofen co-administered intramuscularly in goats is presented as semi logarithmic plot in Figure 1.

On concurrent administration of levofloxacin (4 mg/kg, IV) and ketoprofen (3 mg/kg, IM), the initial plasma concentration of levofloxacin at 2 min was 11.1 ± 0.41 µg/mL, which declined rapidly to 2.32 ± 0.08 µg/mL at 1 h and the drug concentration of 0.015 ± 002 µg/mL was detected up to 18 h. Plasma drug concentration of 11.22 ± 0.42 µg/mL observed at 2 min in febrile goats declined to 2.60 ± 0.11 µg/mL at 1 h, which was significantly higher as compared to plasma drug concentration found at 1 h in normal

goats. Plasma drug concentrations observed were significantly higher from 1 to 18 h in febrile goats than normal goats except at 4 h. The drug (levofloxacin) levels above the minimum inhibitory concentration (MIC: 0.05 μg/mL) were detected in plasma up to 12 h following co-administrated with ketoprofen and in febrile state. Various levofloxacin pharmacokinetic determinants that describe the absorption and elimination pattern after concurrent levofloxacin intravenous administration with ketoprofen intramuscular administration and under febrile state in goats are presented in Table 1.

No adverse effects or toxic manifestations were observed in goats following intravenous administration levofloxacin (4 mg/kg) in concurrent administration with ketoprofen (3 mg/kg, IM). In endotoxin induced febrile state, symptoms viz., increased respiration and pulse rate, decrease in feed intake, dryness of mouth and muzzle, and incoordination in movements were observed.

Following intravenous administration of levofloxacin in ketoprofen-treated goats, no significant changes in pharmacokinetic parameters were observed compared to pharmacokinetic parameters of levofloxacin in normal goats (Patel *et al.*, 2013). Following intravenous administration of levofloxacin in febrile goats, no significant changes were observed with the mean values of important pharmacokinetic parameters viz. Vd_{ss}, $t_{½\alpha}$, $t_{1/2\beta}$, and MRT compared to levofloxacin administration in normal goats. The values of AUC (13.48 ± 0.48 μg.h/mL) and AUMC (40.01 ± 2.66 $\mu g.h^2$/mL) were significantly higher than the values obtained after levofloxacin administration in normal goats (Patel *et al.*, 2013). The value of Cl_B (0.29 ± 0.009 L/h/kg) was significantly lower than the values obtained after levofloxacin administration in normal goats (0.35 ± 0.022 L/h/kg) (Patel *et al.*, 2013).

Following intravenous administration of levofloxacin in febrile goats, the significant decrease in the Clearance (Cl_B) and increase in Area under plasma curve (AUC) may be due to endotoxin induced toxic and adverse effects on the kidneys, including direct vascular damage to the endothelium and platelet aggregation in renal glomerular capillaries. It also produces some functional changes including decrease in the renal blood flow and glomerular filtration rate and changes in the intra-renal hemodynamics (Jernigan *et al.*, 1988, Hasegawa *et al.*, 1999). Endotoxin could produce a metabolic acidosis, which would cause a decrease in urinary pH in febrile animals (Van Miert, 1990) and may favour reabsorption of drug from renal tubules. In addition decrease in systemic vascular pressure, central venous pressure, cardiac output (Salam Abdullah and Baggot, 1986), the peripheral blood flow (due increases in body temperature and to counter act loss of heat), gastrointestinal and hepatic blood flow, which has been reported in ruminants given E. coli endotoxin (Waxman *et al.*, 2003). It is probable that the decrease in glomerular filtration rate induced by endotoxin plays an important role in the decrease of body clearance of drugs which are widely eliminated by the renal route, including levofloxacin. In addition to this the acute phase response induced by febrile state includes synthesis of acute phase hepatic proteins, including α_1-acid glycoprotein, which binds some drugs and may produce a decrease in their clearance as levofloxacin has 22 % degree of plasma protein binding (Goudah and Abo-El-Sooud, 2010).

It is suggested that the critical breakpoints determining the efficacy of fluoroquinolones are C_{max}/MIC_{90} ≥8–10, and $AUC_{0–24}/MIC_{90}$ ≥100 to avoid bacterial resistance emergence (Dudley, 1991; Drusano *et al.*, 1993; Madras-Kelly *et al.*, 1996; Walker, 2000; Toutain *et al.*, 2002).

Fig.1 Semilogarithmic plot of plasma concentrations after intravenous administration of levofloxacin (LFX) (4 mg/kg) in Ketoprofen (KTP)-treated (3 mg/kg) and febrile goats. Each point represents mean of six animals

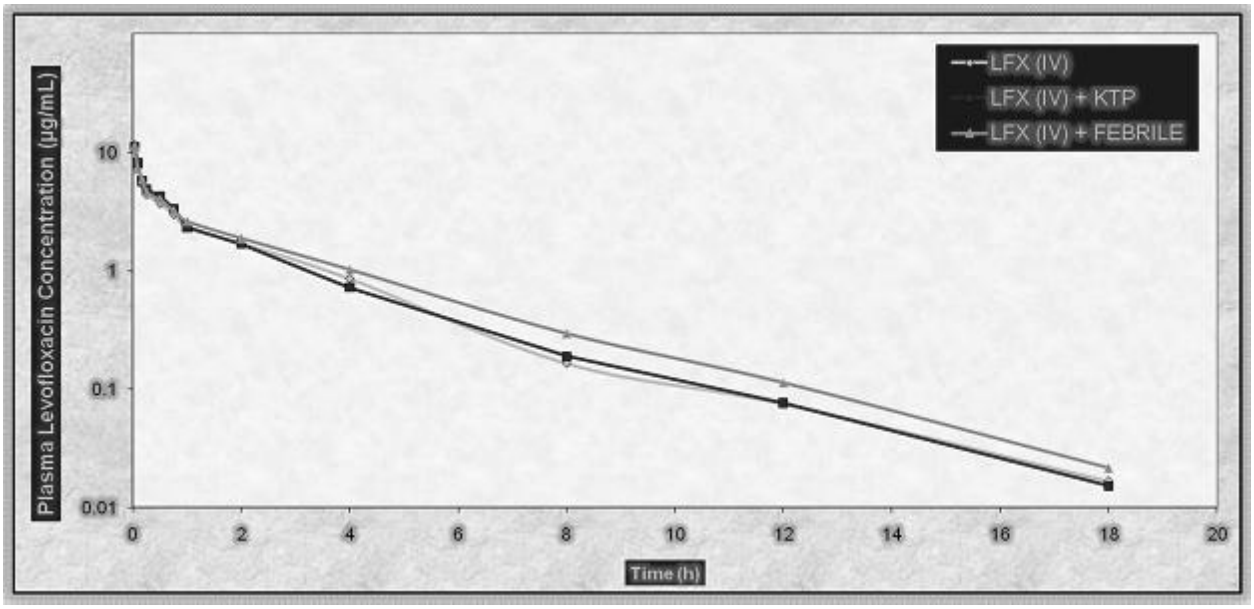

Table.1 Levofloxacin (4 mg/kg) pharmacokinetic parameters following intravenous administration in ketoprofen treated (3 mg/kg) and febrile goats. (Mean ± SE, n=6)

Pharmacokinetic Parameter	Unit	Levofloxacin (IV)	Levofloxacin (IV) and Ketoprofen (IM)	Levofloxacin (IV) and Febrile State
Cp^0	µg/mL	6.68 ± 0.51	7.41 ± 0.61	**6.49 ± 0.63**
A	µg/mL	4.75 ± 0.50	5.54 ± 0.70	**3.37 ± 0.88**
B	µg/mL	1.92 ± 0.29	1.86 ± 0.13	**3.11 ± 0.37**
α	h^{-1}	1.27 ± 0.13	1.29 ± 0.088	**1.31 ± 0.19**
β	h^{-1}	0.27 ± 0.005	0.27 ± 0.004	**0.27 ± 0.003**
$t_{1/2\alpha}$	H	0.57 ± 0.05	0.54 ± 0.03	**0.67 ± 0.20**
$t_{1/2\beta}$	H	2.53 ± 0.05	2.56 ± 0.04	**2.5 ± 0.02**
$AUC_{0-\infty}$	µg.h/mL	11.65 ± 0.71	11.66 ± 0.23	**13.48 ± 0.43***
AUMC	$\mu g.h^2/mL$	30.17 ± 3.99	29.28 ± 1.77	**40.01 ± 2.66***
Vd_{ss}	L/kg	0.86 ± 0.02	0.85 ± 0.02	**0.85 ± 0.03**
Cl_B	L/h/kg	0.35 ± 0.022	0.34 ± 0.006	**0.29 ± 0.009***
MRT	H	**2.53 ± 0.19**	**2.5 ± 0.10**	**2.95 ± 0.13**

*Significant at $p<0.05$ when compared with respective values of intravenous levofloxacin administered alone in goats (Patel *et al.*, 2013).

K_a: Absorption rate constant, B: Zero-time intercept of elimination phase, $t_{1/2ka}$: Absorption half-life, $t_{1/2\beta}$: Elimination half-life, C_{max}: Maximum drug concentration, T_{max}: Time of maximum observed concentration in plasma, $AUC_{0-\alpha}$: Area under curve, AUMC: Area under first moment of curve, Vd_{ss}: Volume of distribution at steady state, Cl_B: Total body clearance, MRT: Mean residence time,

Calculation of surrogate parameter following intravenous administration of levofloxacin (4 mg/kg body weight) in ketoprofen-treated and febrile goats resulted in Cp^0/MIC ratio (MIC: 0.05 µg/ml) of 148.20 and 129.80 respectively, which exceeds the recommended ratio of Cp^0/MIC ratio.

For AUC/MIC ratio at MIC: 0.05 µg/mL and 0.1 µg/mL were found higher than 100 in ketoprofen-treated (233.20 and 116.60) and febrile condition (269.60 and 134.80) respectively. Following intravenous administration, average minimum inhibitory concentration i.e 0.05 µg/ml of levofloxacin maintained in plasma up to 12 h in ketoprofen treated goats and in febrile goats. Considering the values of C_{max}/MIC and AUC/MIC ratios obtained in the present study, it can be concluded that levofloxacin administered intravenous at the dose rate of 4 mg/kg at 12 hr interval may be efficacious against bacteria with MIC values under 0.05 µg/mL satisfactorily.

Levofloxacin can successfully co-administrated with Ketoprofen for combating inflammatory conditions and in febrile condition without alteration of dosage regimen of levofloxacin.

Moreover integrating the pooled pharmacokinetic data generated from the present study, levofloxacin via intravenous administration (4 mg/kg) repeated at 12 h interval is sufficient to maintain plasma concentration above the 0.05 µg/mL MIC for most of the gram-positive and gram-negative microorganisms.

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