



## Influence of *Escherichia coli* lipopolysaccharide induced fever on the plasma kinetics of cefquinome in buffalo calves

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Received: 27 March 2015; Accepted: 22 April 2015

### ABSTRACT

The pharmacokinetic behavior of cefquinome was studied in healthy and febrile buffalo calves after single intramuscular administration (2 mg/kg). The fever was induced with *Escherichia coli* lipopolysaccharide (1 µg/kg, IV). The drug concentration in plasma was detected by HPLC method. Pharmacokinetic analysis of disposition data indicated that intramuscular administration data were best described by one-compartment open model. At 2.5 min, concentrations of cefquinome in healthy and febrile animals were  $2.21 \pm 0.38$  and  $2.52 \pm 0.38$  µg/ml, respectively, and drug was detected up to 24 h. The peak plasma levels of cefquinome in febrile buffalo calves ( $7.70 \pm 0.46$  µg.ml<sup>-1</sup>) and healthy buffalo calves ( $6.70 \pm 0.46$  µg.ml<sup>-1</sup>) were achieved at 45 min and 30 min, respectively. The absorption half life ( $t_{1/2ka}$ ) of cefquinome was similar in healthy and febrile buffalo calves. Drug distribution was altered by fever as febrile animals showed volume of distribution ( $0.37 \pm 0.07$  L/kg) lower than healthy animal ( $0.69 \pm 0.04$  L/kg). Total body clearances in healthy and febrile animals were 0.09 and 0.04 L/kg/h, respectively. To maintain minimum therapeutic concentration of 0.1 mg/ml, a satisfactory dosage regimen of cefquinome in healthy and febrile buffalo calves would be 2.8 mg/kg and 1.2 mg/kg body weight, respectively, to be repeated at 24 h intervals.

**Key words:** Buffalo calves, Cefquinome, Dosage regimen, Fever, Pharmacokinetics

Cefquinome, an aminothiazolyl cephalosporin and a member of the fourth generation of cephalosporins and developed especially for use in animals, has a very broad spectrum of activity against many bacteria (Guerin-Faubleee *et al.* 2003). Cefquinome possesses antibacterial activity against *Pasteurella multocida*, *Mannheimia haemolytica*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus suis*. It is indicated in the treatment of respiratory tract diseases, acute mastitis, calf septicemia and foot rot in cattle, respiratory diseases in pigs and metritis-mastitis-agalactia syndrome in sows, foal septicemia and respiratory tract diseases in horses (CVMP 1995, 1999, 2003). Pharmacokinetics of chemotherapeutic agents are markedly altered in disease conditions (Sharma and Shah 2012, Sharma *et al.* 2014), hence the dosage regimen obtained in healthy subjects cannot be extrapolated in clinical cases to treat diseased animals. Disease states can alter the disposition of a drug to an extent that modification of usual dosage is required for safety and efficiency of the drug. The pharmacokinetic studies of

cefquinome were conducted in sheep (Tohamy 2011, Uney *et al.* 2011), camels (Al-Taher 2010), goats (Dumka *et al.* 2013), healthy buffalo calves (Dinakaran *et al.* 2013) and piglets (Song *et al.* 2013). However, there is paucity of data on the pharmacokinetic study of cefquinome in febrile conditions in buffalo species. Fever, one of the most common manifestations of many infectious diseases, changes heart rate, renal blood flow, hepatic and total splanchnic blood flow, diuresis, enzyme activities and endocrine function (Kasting *et al.* 1982), thereby altering the pharmacokinetics of drugs (Forsyth *et al.* 1982). So, the study on, influence of fever on the pharmacokinetics of antibiotics is essential. The study was conducted to determine the pharmacokinetic variables of cefquinome in febrile buffalo calves following intramuscular administration. From the pharmacokinetic data, recommendations were made for optimal dosage regimen of cefquinome in febrile buffalo calves.

### MATERIALS AND METHODS

The experiments were performed on 6 healthy male buffalo calves of 6–12 months of age and 75–120 kg weight. The animals were acclimatized in the animal shed of department under uniform conditions for 6 weeks before the start of experiment. During this period, all animals were subjected to regular clinical examination. Animals were maintained on green fodder, wheat straw and water *ad lib*.

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Before the start of experiment, permission for experiments on these animals was taken from the Institutional Animal Ethics Committee (IAEC).

Cefquinome sulphate was administered by the intramuscular route at single dose of  $2 \text{ mg.kg}^{-1}$  body weight. Intramuscular administration was performed in the deep gluteal muscle of hind quarter. After 1 month the study was repeated after inducing a febrile state in the same animals. Fever was induced by single/repeated intravenous injection of *E. coli* lipopolysaccharide @  $1 \mu\text{g/kg}$  body weight (Sharma *et al.* 2014). Fever was monitored by measuring the rectal temperature at every 30 min intervals. Once fever was induced cefquinome was injected intramuscularly to these 6 animals @  $2 \text{ mg/kg}$  body weight. Blood samples (4–5 ml each) were withdrawn from the contra lateral jugular vein into heparinized glass test tube before administration and at 2.5, 5, 10, 15, 30, 45 min and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16 and 24 h after administration of drug in each group. Plasma was separated after centrifugation at 2000 rpm for 15 min at room temperature and stored at  $-20^\circ\text{C}$  till analysis usually the next day. Plasma concentrations of cefquinome were determined using high performance liquid chromatography (HPLC) as per Uney *et al.* (2011). The calibration curve for plasma samples was constructed in the range of 0.05 to  $20 \mu\text{g/ml}$ . The limit of detection was  $0.05 \mu\text{g ml}^{-1}$  in plasma. The kinetic parameters were calculated from the formulae derived for a mono compartment open model (Gibaldi and Perrier 1982). The statistical analysis was performed using SPSS® 12.0 software package. The paired t-test was used to compare the parameters obtained in healthy and febrile animals. The dosage regimen (D) of cefquinome was determined based on kinetic data (Baggot 1977) by using following formulae:

$$D = C_p(\text{min}). V_d(e^{\beta t})$$

Where  $C_p(\text{min})$  is the minimum therapeutic concentration of cefquinome,  $t$  is the dosage interval and other parameters are defined in Table 1.

## RESULTS AND DISCUSSION

The administration of *E. coli* lipopolysaccharide induced fever within 2–2.5 h, which persisted for 16 h. At least  $2^\circ\text{F}$  increase of temperature from the normal temperature was taken as the time of cefquinome administration. The mean plasma concentrations of cefquinome as a function of time following intramuscular administration in healthy and febrile animals are shown in Fig. 1.

Evaluation of the results on plasma cefquinome levels against time indicated that pharmacokinetics of cefquinome after its intramuscular administration in healthy and febrile buffalo calves follow mono compartment open model with the exponential equation  $C_p = Be^{-\beta t} - Ae^{-K_a t}$ , where  $C_p$ , cefquinome concentration at time  $t$ ;  $A$  and  $B$ , zero-time intercepts of absorption and elimination phases of the plasma concentration-time curves, respectively;  $K_a$  and  $\beta$ , absorption and elimination rate constants, respectively; and  $e$ , base of natural logarithms.

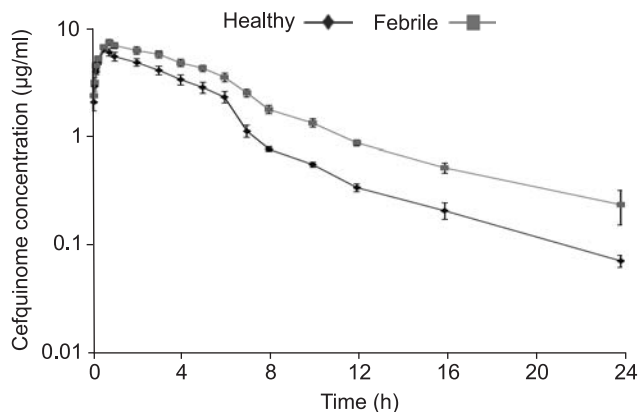


Fig. 1. A semi logarithmic plot of plasma levels time profile of cefquinome after a single intramuscular dose of  $2 \text{ mg kg}^{-1}$  body weight in healthy and febrile buffalo calves. Values given are mean  $\pm$  SE of 6 animals.

The peak plasma levels of cefquinome in febrile buffalo calves ( $7.70 \pm 0.46 \mu\text{g.ml}^{-1}$ ) and healthy buffalo calves ( $6.70 \pm 0.46 \mu\text{g.ml}^{-1}$ ) were achieved at 45 min and 30 min, respectively. At most of the times the plasma concentrations in febrile animals were higher than healthy animals. There was significant difference in these values from 3 to 16 h. Higher blood levels of cefepime in rabbits (Goudah *et al.* 2006, Abd El-Aty 2007), cefuroxime in goats (Prawez and Raina 2006), cephaloridine, oxytetracycline (Singh *et al.* 1997, 1998) and amikacin (Saini and Srivastava 1997) in febrile crossbred cow calves have been reported. The various pharmacokinetic parameters are presented in Table 1.

The values of absorption half life, elimination half life, the average volume of distribution and total body clearance in healthy animals were  $0.12 \pm 0.02 \text{ h}$ ,  $5.29 \pm 0.93 \text{ h}$ ,  $0.37 \pm 0.07 \text{ L.kg}^{-1}$  and  $0.04 \text{ L.kg}^{-1}.\text{h}^{-1}$ , respectively. The absorption half life ( $t_{1/2ka}$ ) of cefquinome was similar in healthy and febrile buffalo calves. Lower value of  $V_{d(\text{area})}$  and  $V_{d(B)}$  in febrile animals indicated that cefquinome is distributed to a lesser extent in various body fluids and tissues of febrile animals as compared to healthy ones. Acute phase response in animals treated with lipopolysaccharides, might have increased plasma acid glycoprotein (AGP) which would increase the plasma protein binding of basic drugs and consequently decrease the distribution of volume of distribution. Further, physical stress induced by endotoxin might alter bodily hydration and the distribution volume of drugs (Van Miert 1990). In accordance to present finding, Saini and Srivastava (1997) reported a decrease in the  $V_{d(\text{area})}$  of amikacin in febrile cow calves as compared to healthy subjects. Changes in the permeability of biological membrane barrier and/or tissue and plasma pH during fever may alter the distribution pattern of drugs. These changes may be more important in patient suffering from infectious encephalitis, prostatitis, arthritis and mastitis. For example in mare with endotoxin-induced arthritis (associated with fever), ampicillin and kanamycin entered into the synovial fluid of inflamed joints more quickly and attained higher

Table 1. Comparative pharmacokinetics of cefquinome following its single intramuscular injection (2 mg.kg<sup>-1</sup>) in healthy and febrile buffalo calves

| Parameters           | Unit                                | Healthy    | Febrile     |
|----------------------|-------------------------------------|------------|-------------|
| A                    | µg.ml <sup>-1</sup>                 | 5.27±0.45  | 6.50±0.89   |
| k <sub>a</sub>       | h <sup>-1</sup>                     | 7.75±1.99  | 7.71±2.24   |
| t <sub>1/2ka</sub>   | h                                   | 0.12±0.02  | 0.12±0.02   |
| AUC                  | µg.ml <sup>-1</sup> .h              | 19.3±0.71  | 40.7±4.31** |
| AUMC                 | µg.ml <sup>-1</sup> .h <sup>2</sup> | 120.7±6.91 | 296.5±65.4* |
| V <sub>d(area)</sub> | L.kg <sup>-1</sup>                  | 0.69±0.04  | 0.37±0.07** |
| V <sub>d(B)</sub>    | L.kg <sup>-1</sup>                  | 0.72±0.05  | 0.38±0.07** |
| B                    | µg.ml <sup>-1</sup>                 | 2.84±0.17  | 6.51±1.02** |
| β                    | h <sup>-1</sup>                     | 0.15±0.01  | 0.15±0.02   |
| t <sub>1/2β</sub>    | h                                   | 4.54±0.21  | 5.29±0.93   |
| Cl <sub>B</sub>      | L.kg <sup>-1</sup> .h <sup>-1</sup> | 0.09±0.00  | 0.04±0.00** |
| MRT                  | h                                   | 5.72±0.17  | 7.21±1.29   |

Values given are mean±SE of the results obtained from six animals. Fever was experimentally induced with intravenous injection of *E. coli* lipopolysaccharide (1 µg.kg<sup>-1</sup>); \*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.

A, B, zero-time plasma drug concentration intercepts of regression lines of absorption and elimination phases, respectively; k<sub>a</sub> and b, absorption and elimination rate constants, respectively; t<sub>1/2ka</sub>, absorption half life; t<sub>1/2β</sub>, elimination half life; AUC, total area under plasma drug concentration-time curve; AUMC, area under the first moment of the plasma drug concentration-time curve; V<sub>d(area)</sub>, apparent volume of distribution, based on area under curve; V<sub>d(B)</sub>, apparent volume of distribution, based on zero-time plasma concentration intercept of the least square regression line of elimination phase; Cl<sub>B</sub>, total plasma clearance; MRT, mean residence time.

concentration than uninflamed joints (Firth *et al.* 1988).

The significantly higher AUC, area under the first moment of plasma concentration-time values in febrile buffalo calves observed in this study showed that the drug remains in the body for a comparatively longer duration in the febrile conditions. Comparison of total body clearance in febrile animals with that of healthy animals revealed that the Cl<sub>B</sub> (0.04 L.kg<sup>-1</sup>.h<sup>-1</sup>) in febrile animals was significantly (P<0.01) different as compared to healthy animals (0.09 L.kg<sup>-1</sup>.h<sup>-1</sup>). The fact that Cl<sub>B</sub> is a function, whose value depends upon elimination half-life and volume of distribution and V<sub>d(area)</sub> decreased in febrile conditions, could be the reason for decreased Cl<sub>B</sub> value of cefquinome in febrile animals. The renal clearance of drug is blood flow dependent, so (i) elimination by the kidney can be impaired when reduced cardiac output compromises renal blood flow (Blatteis *et al.* 1988) and (ii) during infectious diseases along with fever, the liver and kidney often show biochemical and pathological evidence of tissue damage and therefore, the possibilities of impaired drug metabolism and excretion arises. Endotoxin induces toxic and adverse effects on the kidneys, including direct vascular damage to the endothelium and platelet aggregation in renal glomerular capillaries. Renal tubular cell injury may be the result of

direct injury by the lipopolysaccharide (Jernigan *et al.* 1988). It also produces some functional changes including a decrease in the renal blood flow and glomerular filtration rate and changes in the intrarenal hemodynamics (Jernigan *et al.* 1988, Hasegawa *et al.* 1999). The arachidonic acid metabolites, thromboxane, prostaglandins, leukotrienes, platelet activating factor and/or endothelin mediated vasoconstriction could result into decrease in glomerular filtration rate. Moreover, it was reported that endotoxin produces an increase in tubular re-absorption and a decrease in tubular secretion of some drugs. The increase in drug re-absorption could be the result of its binding to negatively charged phospholipids in the renal brush border membrane surface, by the presence of negatively charged endotoxin (Hasegawa *et al.* 1999). Further these points would be more pertinent for cephalosporins specially cefquinome, which are mainly excreted by renal route.

The acute phase response (APR) is defined as a pathophysiological condition induced by many causal factors, i.e. infection, inflammation and tissue. The APR induces many systemic changes, which include fever, increased lassitude, loss of appetite as well as the synthesis and secretion of acute phase hepatic proteins (Van Miert 1991). Significant concentrations of proinflammatory cytokines, such as tumor necrosis factor-α, interleukins and interferons, are produced during APR, which leads to the direct suppression of the microsomal cytochrome P450 (CYP)-dependent activity in the liver (Wright and Morgan 1991, Chen *et al.* 1992, Abdel-Razzak *et al.* 1993). This might in turn alter the pharmacokinetic profile of the some drug.

The main objective of present study was to determine a satisfactory dosage regimen of cefquinome in febrile buffalo calves. It is not axiomatic to compute the dosage regimen of cefquinome to be used effectively in clinical practice for the treatment of mild to severe bacterial infections, without having first conducted a detailed pharmacokinetic study. With minimum therapeutic plasma concentration of cefquinome as 0.1 mg/ml which has been shown to be most effective against the majority of sensitive Gram-positive and Gram-negative pathogens, the convenient and suitable dosage regimen of cefquinome in healthy and febrile animals was 2.8 mg/kg and 1.2 mg/kg, respectively, repeated at 24 h intervals.

On the basis of present study, lipopolysaccharide induced fever markedly altered the dosage regimen of cefquinome in buffalo calves, resulting the dosage obtained in healthy animal needs to be modified. By doing so, the therapeutic efficacy of cefquinome may be increased and the adverse effects related to drug as well as the cost factor of treatment may decrease.

#### ACKNOWLEDGMENT

The financial assistance provided by Council of Scientific and Industrial Research, New Delhi (India) in the form of research grant (Scheme No: 37(1395)/10/EMR-II) is gratefully acknowledged.

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