



## Pharmacokinetics and testing of dosage regimen of cefazolin post intravenous injection in healthy female buffalo calves

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Cefazolin is a semi-synthetic first generation cephalosporin having good activity against most gram-positive bacteria and against some gram-negative bacteria such as *Escherichia coli*, *Salmonella* and *Pasteurella* species. Cefazolin achieves higher plasma and bone concentrations and has a longer half-life in many species than other first generation cephalosporins (Carpile 1988). The study was undertaken to know the distribution of cefazolin in biological fluids in healthy female buffalo calf and to calculate the dosage regimen, viz. loading or priming dose ( $D^*$ ) and maintenance dose ( $D_0$ ) following single i/v administration. Further, in the present study these calculated doses were administered at selected dosages interval ( $\gamma$ ) repetitively for 3 times. The dosage regimen was tested by measuring the concentration of antimicrobial (cefazolin) at different time during the repetitive administration of the drug to know whether the minimum inhibitory concentration (MIC) is maintained or not?

**Experimental animals and route of administration:** In the present study kinetics of cefazolin was carried out in 4 she buffalo calves weighing between 120 and 180 kg body weight under healthy condition. Pure cefazolin was injected as a single dose (10 mg/kg) intravenously in left jugular vein.

**Collection of biological samples:** Samples of plasma were collected at different time intervals i.e. 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h initially from right jugular vein and then alternately from both left and right jugular vein in heparinized centrifuged tubes for plasma and glass tubes for urine. For collection of urine a sterile balloon catheter (No. 12) was introduced through urethra and held in position by injecting water (around 15–20 ml) in each female buffalo calf. Samples were kept under deep freeze at  $-20^\circ\text{C}$ , and were analyzed usually within 48 h.

**Method of estimation:** Drug concentrations in biological samples (plasma and urine) were estimated by

microbiological assay technique (cylinder plate diffusion) using *Sarcina lutea* (ATCC 9341) as the test microorganism (British Pharmacopoeia 1980). The test samples were used along with standards and triple assay were carried out. The limit of quantification (LOQ) is  $\leq 0.01 \mu\text{g/ml}$ .

**Calculation of kinetic parameter:** Kinetic parameters were calculated on the basis of Baggot (1977), Notari (1982) and Gibaldi and Barrier (1975, 1982). Based on kinetic parameters, dosage regimen was derived using the following formula.

For calculation of  $D^*$  (loading or priming dose) and  $D_0$  the following formulae of Baggot (1977) were used

$$D^* = C_p (\text{min}). V_{d_{\text{area}}} (e^{\beta \cdot \gamma})$$

$$D_0 = C_p (\text{min}). V_{d_{\text{area}}} (e^{\beta \cdot \gamma} - 1)$$

where,  $C_p^\infty$  (min), minimum therapeutic plasma drug concentration;  $V_{d_{\text{area}}}$ , volume of distribution based on total area under the plasma drug concentration versus time curve;  $\beta$ , elimination rate constant;  $\gamma$ , dosage interval;  $e$ , base of natural logarithm.

**Calculation of dosage regimen:** Dosage regimen was calculated as per Baggot (1977). For carrying out testing of dosage regimen 4 buffalo calves were used and calculated dosage regimen of cefazolin were used for repetitive administration for maintaining  $C_p^\infty$  min (MIC) of  $0.5 \mu\text{g/ml}$  at dosage interval ( $\gamma$ ) of 6 h with 1 loading dose and 3 maintenance doses following i/v administration. The statistical analysis of all the data was done by Student's  $t$  test as per Snedecor and Cochran (1967).

The log plasma drug concentrations followed biphasic curve and hence all kinetic parameters were derived accordingly. The study revealed concentration of cefazolin in plasma and urine at various time intervals after a single i/v administration @ 10 mg/kg. The drug was present up to 10 h in plasma. The drug concentrations were higher up to 30 min and thereafter lower concentrations were noted. The drug was present in therapeutic concentration ( $\geq 0.5 \mu\text{g/ml}$ ) up to 4 h in plasma and  $> 12$  h in urine, respectively. The drug reached its peak concentration in plasma ( $C_p \text{ max}$ ) of  $13.06 \pm 0.60 \mu\text{g/ml}$ . The drug reached its peak concentration in urine ( $C_U \text{ max}$ ) of  $1,139 \pm 277.6 \mu\text{g/ml}$  at

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20 min. In urine, therapeutic concentration was maintained up to 12 h.

Various kinetic parameters obtained by 2-Compartment open model since log plasma drug concentrations *versus* time profile showed a biphasic pattern are given in Table 2. It showed a short  $t_{1/2\alpha}$  of  $0.181 \pm 0.028$  h and a longer  $t_{1/2\beta}$  of  $4.10 \pm 0.57$  h that revealed a rapid distribution but a slower elimination in female buffalo calves. In contrast, short  $t_{1/2\beta}$  of 1.44 h (Barth and Weinstein 2011), 1.8 h (Baggot 2001) and 2.0 h (Lee *et al.* 1980) in man, 0.65 h in horse (Baggot 2001), 0.8 h in dog (Baggot 2001),  $1.18 \pm 0.14$  h in goat (Varun Ahuja *et al.* 2009) and  $\beta$  of 0.44 h<sup>1</sup> in human and  $t_{1/2\beta}$  1.6 h rat (Woodnut *et al.* 1992) were reported. The above noted data revealed that the drug is distributed faster but removed from the body of buffalo calf slowly than the other species.

A high volume distribution ( $V_{d\text{ area}}$ ) of  $6.06 \pm 0.73$  L.kg<sup>-1</sup> was obtained for buffalo calf in the present study as compared to 0.4 L.kg<sup>-1</sup> in rat, 0.1 L.kg<sup>-1</sup> in rabbit, 0.7 L.kg<sup>-1</sup> in dog and 0.2 L.kg<sup>-1</sup> in human (Lee *et al.* 1980). A high volume of distribution < 1 L.kg<sup>-1</sup> indicates apart from its wide distribution, it may be metabolized or excreted in urine (Baggot 1977). In the present study, a high amount of excretion of the drug in urine was noted at various time intervals (Table 1). Renal clearance of 80% in rat, 90% in rabbit, 80% in dog and 90% in human was noted as % dose excreted in 48 h (Lee *et al.* 1980).

Total body clearance ( $Cl_B$ ) of  $17.66 \pm 2.44$  ml.kg<sup>-1</sup>.min<sup>-1</sup> was observed in the present study for buffalo calf. However, a lower  $Cl_B$  of 9.7 ml.kg<sup>-1</sup>.min<sup>-1</sup> in rat, 5.3 ml.kg<sup>-1</sup>.min<sup>-1</sup> in rabbit, 10.4 ml.kg<sup>-1</sup>.min<sup>-1</sup> in dog and 0.7 ml.kg<sup>-1</sup>.min<sup>-1</sup> in human were noted (Lee *et al.* 1980). However, a higher  $Cl_B$   $3.36 \pm 0.12$  L.kg<sup>-1</sup>.h<sup>-1</sup> (56 ml.kg<sup>-1</sup>.min<sup>-1</sup>) was noted in healthy goat (Varun Ahuja *et al.* 2009).

Table 1. Concentrations (µg/ml) of cefazolin in she buffalo calf under healthy condition after a single i/v dose of 10 mg/kg

| Time (h) | Value (mean±sem) n = 4 |             |
|----------|------------------------|-------------|
|          | Plasma                 | Urine       |
| 0.083    | 13.06±0.60             | 83.60±37.58 |
| 0.167    | 7.92±1.06              | 220.1±93.53 |
| 0.25     | 4.17±0.18              | 706.3±191.7 |
| 0.333    | 3.29±0.24              | 1139±277.6  |
| 0.50     | 2.78±0.32              | 776.8±242.5 |
| 0.75     | 2.00±0.24              | 585.5±235.1 |
| 1.0      | 1.50±0.16              | 423.5±138.3 |
| 1.5      | 1.21±0.12              | 362.5±130.7 |
| 2.0      | 0.78±0.13              | 252.6±56.67 |
| 3.0      | 0.59±0.10              | 179.8±49.99 |
| 4.0      | 0.47±0.07              | 114.4±43.35 |
| 5.0      | 0.40±0.06              | 84.05±34.34 |
| 6.0      | 0.33±0.08              | 46.83±12.70 |
| 8.0      | 0.17±0.10              | 35.08±8.11  |
| 10       | 0.08±0.08              | 14.34±3.34  |
| 12       | N. D                   | 2.95±1.14   |
| 24       | —                      | ND          |

ND, Non detectable.

Table 2. Kinetic parameters of cefazolin calculated by 2-compartment open model after a single i.v injection (10 mg/kg) under healthy condition in she buffalo calf

| Parameter (Unit)                                          | Mean ± sem (n=4) |
|-----------------------------------------------------------|------------------|
| A (µg/ml)                                                 | 11.45±4.20       |
| B (µg/ml)                                                 | 1.03±0.21        |
| C <sub>0</sub> <sup>P</sup> (µg/ml)                       | 12.48±4.22       |
| α = (h <sup>-1</sup> )                                    | 2.668±0.709      |
| t <sub>1/2</sub> α = (h)                                  | 0.181±0.028      |
| β = (h <sup>-1</sup> )                                    | 0.31±0.06        |
| t <sub>1/2</sub> β = (h)                                  | 4.10±0.57        |
| AUC (mg/L.h)                                              | 9.93±1.29        |
| AUMC (mg/L.h <sup>2</sup> )                               | 38.02±9.82       |
| MRT (h)                                                   | 3.62±0.55        |
| K <sub>12</sub> (h <sup>-1</sup> )                        | 1.270±0.467      |
| K <sub>21</sub> (h <sup>-1</sup> )                        | 0.394±0.065      |
| Kel (h <sup>-1</sup> )                                    | 1.184±0.249      |
| F <sub>C</sub>                                            | 0.48±0.05        |
| T ≈ P                                                     | 6.44±2.44        |
| V <sub>d area</sub> (L.Kg <sup>-1</sup> )                 | 6.06±0.73        |
| Cl <sub>B</sub> (ml.kg <sup>-1</sup> .min <sup>-1</sup> ) | 17.66±2.44       |

A, zero time intercept for distribution phase; B, zero time intercept for elimination phase; C<sub>0</sub><sup>P</sup> (µg/ml), theoretical zero time concentration (A+B); α, distribution rate constant; t<sub>1/2</sub> α, distribution half life; β, elimination rate constant; t<sub>1/2</sub> β, elimination half life; AUC, total area under plasma drug concentration curve; AUMC, area under first moment curve; MRT, mean residential time; K<sub>12</sub>, rate constant of drug transfer from central compartment to peripheral compartment; K<sub>21</sub>, rate constant of drug transfer from peripheral to central compartment; Kel, rate constant of drug elimination from central compartment; F<sub>C</sub>, fraction of drug available for elimination from central compartment; T ≈ P, approximate tissue to plasma concentration ratio; V<sub>d area</sub>, apparent volume of distribution; Cl<sub>B</sub>, total body clearance.

Most of the kinetic parameters of cefazolin obtained in the present study for buffalo calf differed from other species. Variations among species, breed, sex and age may contribute to the wide discrepancies in kinetic parameters (Jayachandran *et al.* 1990, Baggot 2001).

It is noted that for *E.coli*, *Salmonella* spp. and other enterobacteriaceae 0.5 to 1 µg/ml of blood/tissue level was taken as susceptible for testing of dosage regimen (Reller and Weinstein 2011). Fig.1 represents the mean D\* and D<sub>0</sub> of 4.91 mg/kg and 2.79 mg/kg of cefazolin at γ of 6 h for 3 consecutive doses for maintaining therapeutic concentration (C<sub>P</sub><sup>∞</sup> min = MIC) of 0.5 µg/ml for treating systemic infections. Similarly, the dosage regimen (D\* and D<sub>0</sub>) can be calculated at γ of 8/12 h for treating systemic infections in buffalo calf.

## SUMMARY

Pharmacokinetics and testing of dosage regimen of cefazolin was carried out in 4 healthy female buffalo calves after its intravenous (iv) administration @ 10 mg/kg. Plasma

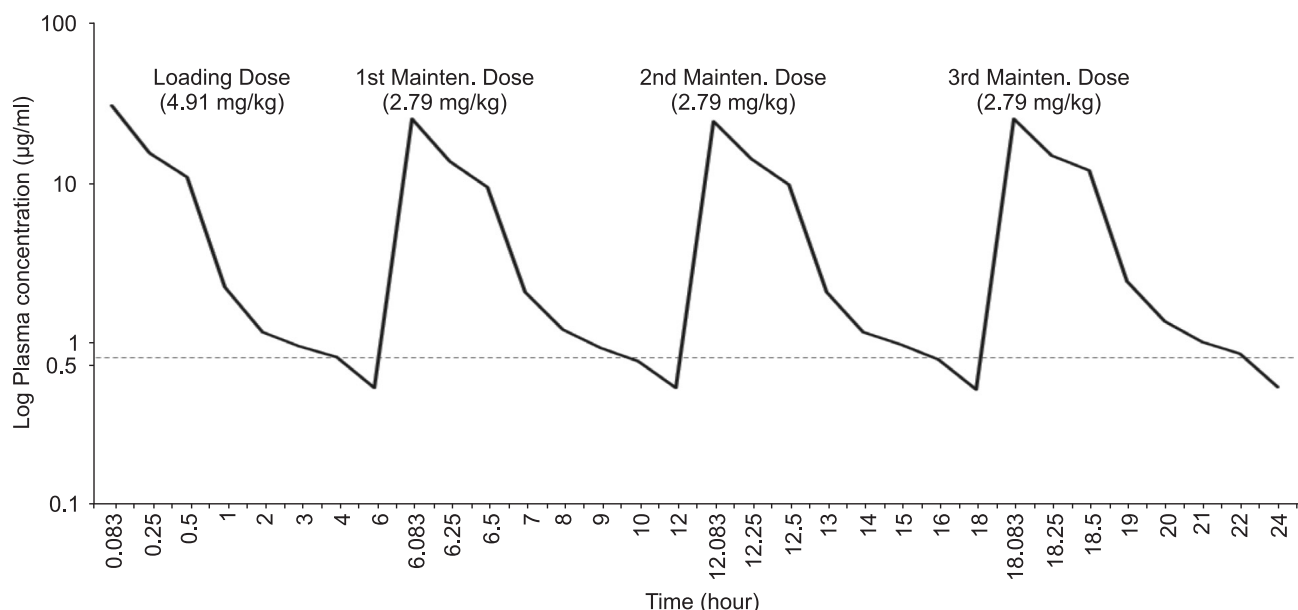


Fig. 1. Plasma concentrations ( $\mu\text{g/ml}$ ) of cefazolin during repetitive administration to verify the maintenance of MIC of  $0.5 \mu\text{g/ml}$  at the dosage interval of 6 h.

and urine concentrations of cefazolin were estimated at various time intervals by microbiological assay using *Sarcina lutea* (ATCC 9341). From log plasma concentrations versus time, kinetics parameters were calculated. Based on kinetic parameters, loading ( $D^*$ ) and maintenance ( $D_0$ ) doses were calculated for maintaining  $C_p^{\infty} \text{ min}$  (MIC) of  $0.5 \mu\text{g/ml}$  at the dosage interval ( $\gamma$ ) of 6 h. Using the data, appropriate  $D^*$  was injected i/v and 3 consecutive  $D_0$  were given at selected dosage interval ( $\gamma$ ) of 6 h to maintain  $C_p^{\infty} \text{ min}$  of  $0.5 \mu\text{g/ml}$  in each animal. The study obtained distribution ( $t_{1/2 \beta}$ ) and elimination ( $t_{1/2 \alpha}$ ) half life, volume distribution, and total body clearance ( $Cl_B$ ) of  $0.19 \pm 0.06 \text{ h}$ ,  $4.78 \pm 0.28 \text{ h}$ ,  $5.49 \pm 0.83 \text{ L/kg}$  and  $13.15 \pm 1.53 \text{ ml/kg/min}$ .  $D^*$  and  $D_0$  of  $6.52 \pm 0.77$  and  $3.77 \pm 0.38 \text{ mg/were}$  calculated to maintain  $C_p^{\infty} \text{ min}$  of  $0.5 \mu\text{g/ml}$  at  $\gamma$  of 6 h.

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