Kinetics and Penetration into Inflammatory Tissue Cage Fluid of Cefepime Administered to Rabbits

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Summary

The kinetics and penetration of cefepime into inflammatory tissue cage fluid were determined in rabbits. Ten adult healthy rabbits were used. Concentrations of cefepime were measured in serum and induced inflammatory exudate by biological methods. The kinetic analysis was performed by mean of a non-compartmental model. Pharmacokinetic results in serum (S) and inflammatory tissue cage fluid (ITCF) (means ± standard error) were: half life of elimination [$t_{1\backslash 2}$ (S)]= 1.6 ± 0.2 and (ITCF) 3.7 ± 0.3 h; area under the curve [AUC (S)]= 225.3 ± 21.4 and (ITCF) 208.0 ± 13.6 (µg/ml/h]; maximum concentration [C_{max} (ITCF)]= 37.7 ± 3.6 µg/ml and time to reach C_{max} [t_{max} (ITCF)]= 1.8 ± 0.4 h and the penetration into inflammatory tissue cage fluid [P (ITCF)] = 92.3 ± 8.7 %. In conclusion, the cefepime administered to rabbits penetrate rapidly and nearly completely into inflammatory tissue cage fluid.

Introduction

Cefepime, a 4th generation cephalosporin, has a wider antibacterial spectrum than most 3rd generation cephalosporins. Although the kinetics of cefepime have been described in humans (*Nye et al., 1989; Reed et al., 1997*) and animals (*Forgue et al., 1987; Guglick et al., 1998; Gardner & Papich, 2001; Ismail, 2005a; Ismail, 2005b*), data for penetration into inflammatory exudate in rabbits is unknown. The aim of the present work was to determine the kinetics of cefepime and measure the penetration into inflammatory tissue cage fluid (ITCF) in rabbits.

Materials and Methods

Ten male New Zealand White rabbits (3.0 to 3.5 kg each) were obtained from the Department of Introduction to Animal Production, Faculty of Agricultural and Forestry Science. Animals were kept in individual stainless cages (50 cm x 50 cm x 60 cm) with perforated floors on a climate-controlled environment (temperature from 16 to 20ºC, relative humidity from 45 to 65% and 12 h light/dark cycles); they were fed with commercial diet (Ganave Conejos®. La Plata, Argentina) and received water *ad libitum*. The animals were maintained and handled in accordance with the NIH guide for the care and use of laboratory animals (*National Research Council, 1985*). The research protocol was approved by the Commission of Scientific Research of the Province of Buenos Aires.

After a week animals were implanted with cages for the collection of tissue cage fluid (TCF). Each animal had six cages implanted subcutaneously, three in the left region and three in the right region. The cages consisted of Silastic (Dow Corning, Midland, MI) rubber tubes, 30 mm long and 0.5 mm inner diameter, closed at one end, with 40% of its surface perforated by 1.0 mm holes. For this purpose, the flank and rib areas of the animals were shaved, washed and disinfected. Implantation sites were anesthetized and cages were inserted in a tunnel bluntly dissected through a skin incision in the areas mentioned above. The animals were kept in individual cages, received water ad libitum, and were fed with commercial diet throughout the trials. Tissue cage

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fluid and blood/serum samples were collected once a week for four weeks after the implant. Samples were analyzed for total proteins by the Biuret method and albumin by binding sulfobromophthalein in order to determine the reaction to the implanted material. Tissue fluid granulocytes were counted previously and 24 hours postadministration of Carrageenan.

The trial was conducted 4 weeks after the implantation of the tissue cages. Rabbits (n= 10) with normal body temperature received in each cage 0.2 ml Carrageenan (Sigma Chemical Company) prepared in 2% physiologic solution in order to produce a local acute inflammatory reaction. 24 hours post-administration of Carrageenan, a single dose of 20 mg/kg b.w. of cefepime (Cefimen K, Klonal) was administered intravenously (left Saphenous vein). Blood samples were obtained by direct venipuncture of the Saphenous vein immediately before medication and at 0.08, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 hours post-administration of the antibiotic. Inflammatory tissue cage fluid samples (0,2 to 0,4 ml each) were also collected following the same schedule, but starting at 0.25 hours post-administration of the drug. Blood samples were allowed to clot and then centrifuged at 3000g for 15 min in order to separate the serum; the samples were stored in individual, sterile recipients at -18°C until being analyzed.

The concentration of cefepime in serum and ITCF was measured using a microbiological assay method (*Bacillus stearothermophilus* var. calidolactis) (*Herbst, 1982*). The correlation coefficients for the regression lines of the standard solutions were not lower than 0.99. The coefficients of variation intra and inter assays were lower than 8 % and the limit of quantification (LOQ) was 0.2 µg/ml.

The percentage of protein binding was calculated by comparing the antimicrobial activity of cefepime in the presence and absence of proteins. For that purpose, standard graphs of microbial inhibition by cefepime in plasma and buffer (at concentrations of 150 and 50 µg/ml) were compared (*Craig & Suh, 1986*).

Pharmacokinetic analysis: the cefepime serum and ITCF concentration data were analysed by a non-compartmental model with WinNonlin software (Pharsight Corp. Mountain View, Calif.). The following pharmacokinetic parameters were determined: elimination half-life ($t_{½}$), area under the curve (AUC), maximum concentration (C_{max}) in ITCF, time to reach C_{max} (t_{max}) and the percent penetration (P) into ITCF [$P= AUC_{(ITCF)}/AUC_{(serum)}$].

The pharmacokinetic and biochemical results were analyzed by means of variance analysis (ANOVA). The level of significance was 5%.

Results

At 4 weeks post-implantation of the cages to collect tissue fluid the average protein concentration in TCF was 48 % of that obtained in serum samples.

Before and 24 hours after the administration of Carrageenan the biochemical results (mean ± standard deviation) in the TCF and ITCF were: total proteins= 2.80 ± 0.34 and 3.08 ± 0.75 g/dl (P>0.05), albumins= 1.82 ± 0.22 and 1.57 ± 0.58 g/dl (P>0.05), globulins= 1.02 ± 0.18 and 1.52 ± 0.37 g/dl (P< 0.05), albumin/globulin ratio= 1.78 ± 0.43 and 1.08 ± 0.44 % (P< 0.05), respectively.

The administration of Carrageenan produced an acute inflammation into the implanted cage. The granulocyte counts in the TCF and ITCF (mean ± standard deviation) at time zero and 24 h were:

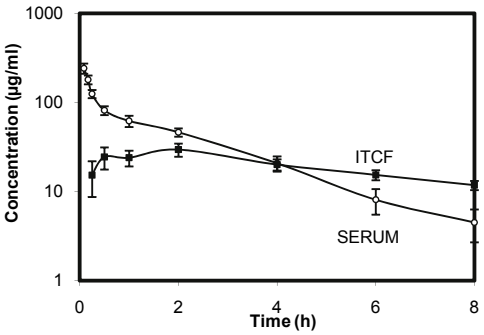

Figure 1. Semi-logarithmic plot of cefepime concentration (Means ± S.D.) in serum and inflammatory tissue cage fluid (ITCF) versus time after intravenous administration to rabbits.

Table 1. Cefepime kinetics in serum and inflammatory tissue cage fluid (ITCF) (Mean ± S.E.) following intravenous administration of 20 mg/kg in rabbits.

Pharmacokinetic Parameters (Units).	*Mean ± S.E.*
$t_{½}$ (h) (serum)	1.6 ± 0.2
AUC (µg/ml/h) (serum)	225.3 ± 21.4
$t_{½}$ (h) (ITCF)	3.7 ± 0.3
C_{max} (µg/ml) (ITCF)	37.7 ± 3.6
t_{max} (h) (ITCF)	1.8 ± 0.4
AUC (µg/ml/h) (ITCF)	208.0 ± 13.6
Penetration (%) (ITCF)	92.3 ± 8.7

($t_{½}$) elimination half-life
(AUC) area under the curve
(C_{max}) maximum concentration
(t_{max}) time to reach C_{max}

1,633 ± 1,565 and 28,650 ± 14,562 x 10^3 cells/ml ($P< 0.05$), respectively.

The concentration-time curves and the pharmacokinetic variables in serum and ITCF of cefepime in rabbits are shown in Figure 1 and Table 1, respectively.

Discussion

Though the concentration-time curves of cefepime have been analyzed mainly by means of a two-compartmental open model (*Guglick et al., 1998; Gardner & Papich, 2001; Ismail, 2005a; Ismail, 2005b*) in our work, like *Reed et al.* (*1997*) we used non-compartmental models.

The half-life of cefepime in serum (1.6 h) was similar to those observed in monkeys (1.7 h) (*Forgue et al., 1987*), ewes (1.76 h) (*Ismail, 2005b*), adult dogs (1.65 h) (*Gardner & Papich, 2001*) and infants and children (1.7 h) (*Reed et al., 1997*) and lower than those obtained in adult humans (approx. 2.0 h) (*Nye et al., 1989*), horses (125.7 min) (*Guglick et al., 1998*) and calves (2.38 h) (*Ismail, 2005a*).

The half-life of cefepime was 2.3 times longer in ITCF ($t_{½}$= 3.7 h) than in serum ($P< 0.05$).

Cefepime penetrates rapidly and in high concentrations into ITCF (t_{max} 1.8 h and C_{max} 37.7 µg/ml, respectively), due to its low binding to plasma proteins (8.0 ± 2.5 %) that would allow the complete diffusion of the antibiotic to the extracellular compartment. *Guerrero & MacGregor* (*1979*) observed an inverse correlation between the penetration of the cephalosporins into inflammatory exudate and their degree of binding to plasma proteins.

The area under the curve in ITCF was similar to that obtained in serum (208.0 and 225.3 µg/ml/h, respectively), thus showing a high penetration into ITCF (nearly 100 %) of the antibiotic. This degree of penetration was higher than that observed with other cephalosporins in inflammatory exudate in rabbits [cephradine (28.5%), cephacetrile (27.7%), cephalothin (14%) and cefamandole (12.5%) (*Guerrero & MacGregor, 1979*).

Conclusion

In conclusion, the administration of an irritating chemical substance in the implanted cages to rabbits, mimicked a local inflammation and resulted in rapid and nearly complete penetration of cefepime into the tissue cage fluid.

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