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Bioequivalence Evaluation of Two Cefquinome 2.5% Injectable Products in Piglets

In-Bae Song, Tae-Won Kim, Hong-Gee Lee, Myoung-Seok Kim*, Youn-Hwan Hwang, Byung-Kwon Park, Jong-Hwan Lim***† and Hyo-In Yun[1]**

Lab of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Chungnam National University, Daejon 305-764, Korea
**Jeollanamdo Development Institute for Traditional Korean Medicine, Jangheung-gun 529-851, Korea*
***Korea Institute of Oriental Medicine, Daejon 305-811, Korea*
****B&C Biopharm, Advanced Institutes of Convergence Technology, Suwon-si 443-759, Korea*

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Abstract : Cefquinome, a fourth generation cephalosporin, has been solely used for veterinary medicine and has a broad antibacterial spectrum against gram-negatives and gram-positives being very stable to β-lactamases. This study was conducted to evaluate the bioequivalence of two cefquinome 2.5% products in piglets. Plasma cefquinome concentrations were analyzed by liquid chromatography-mass spectrometry (LC/MS). Mean maximum concentration (C_{max}) of test product (Cequus®) and reference product (Cobactan®) were 4.34 ± 0.58 and 4.22 ± 0.47 μg/mL, and mean area under the concentration time curve ($AUC_{0\rightarrow\infty}$) values were 10.43 ± 1.96 and 10.25 ± 2.98 μg·h/mL, respectively. The 90% confidence intervals for the ratio of C_{max} (0.941-1.115), and $AUC_{0-\infty}$ (0.927-1.172) values for the test and reference products were within the acceptable bioequivalence limit of 0.80-1.25. It is concluded that two commercial cefquinome injectable solutions are bioequivalent in their extent of drug absorption in piglets.

Key words **:** bioequivalence, cefquinome, LC/MS, pharmacokinetic.

Introduction

Bioequivalence of the two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. While the area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption, the peak concentration (C_{max}) and the time of its occurrence (T_{max}) reflect the rate of absorption (7).

Cefquinome,1-[(6*R*,7*R*)-7-[[(2*Z*)-(2-amino-4-thiazolyl)-(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0-oct-2-en-3-yl]methyl]-5, 6, 7, 8-tetrahydroquinolinium inner salt, is a new fourth generation aminothiazolyl cephalosporin and was developed solely for veterinary use (2). In comparison with third generation cephalosporins, it is more susceptible to gram-negative bacteria and has a lower affinity for plasmid-mediated cephalosporinases including AmpC type (13). It has been approved for the treatment of respiratory tract diseases, acute mastitis and foot rot in cattle, calf septicemia, respiratory diseases in pigs and metritis-mastitis-agalactia syndrome in sow (2,3). The pharmacokinetics of cefquinome have been reported for bovine, camels, sheep, ducks, and piglets (1,4,5,10,14), showing a rapid absorption, low protein binding property and good bioavailability.

There are various cefquinome injectable solutions formulated with different excipients available on the market, which are sold under different brand names, but contain the same active substance at the same strength. Vehicle components used in different products could change the pharmacokinetics of cefquinome in animals. In the present study, two different injectable commercial preparations containing the same amount of cefquinome were carried out to evaluate their bioequivalence following intramuscular (i.m.) injection in healthy piglets.

Materials and Methods

Chemicals

The analytical standards of cefquinome and ceftiofur (internal standard, IS) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich Inc. (St Louis, USA), respectively. The commercial injectable preparations of cefquimone sulfate, Cobactan® (cefquinome sulphate 2.5%, Batch No. A346AO1, Intervet International, Unterschleissheim, Germany) as reference product and Cequus® (cefquinome sulphate 2.5%, Batch No. 090528, Woogene B&G, Hwasung, Korea) as test product, were purchased from local distributors. HPLC grade acetonitrile (ACN), methylene chloride and trifluoroacetic acid (TFA) were obtained from J.T. Baker

†These two authors contributed equally to this work
[1]Corresponding author.
E-mail : hiyun@cnu.ac.kr.

(Deventer, Netherlands).

Animals and experimental design

This study was performed in 12 healthy cross-bred (Landrace × Duroc × Yorkshire) piglets weighing 23-26 kg and aged about 7 weeks. They were fed with antibiotic-free diet and provided with groundwater *ad libitium* throughout the period.

The study was conducted with a randomized, open-label, single dose, two-period crossover design. The animals were divided into two groups of 6 animals each. One group was i.m. injected into the gluteal muscles with the reference product (Cobactan®) at 2 mg/kg and the other group was treated with the test product (Cequus®) at the same dose and the same route, and vice versa with 7 days of washout period. In both experimental periods, all animals were fasted 12 h prior to injection. Blood samples were collected from the jugular vein at 0 (pre-), 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8 and 12 h following i.m. injection of cefquinome. The samples were taken in heparinized tubes and plasma was obtained by centrifugation. Plasma samples were stored at −70°C until analysis. All procedure of experiment was approved by the Animal Ethics Committee, Chungnam National University (Daejeon, Korea).

Sample preparation and analysis

Plasma samples (100 μL) were added 10 μL of IS (1 μg/ml) and 200 μL ACN. The samples were vortexed for 5 min and then centrifugated at 10,000 *g* for 10 min. After centrifugation, the supernatant was transferred to another tube followed by adding 600 μL of methylene chloride. After vortexing for 15s, the sample was centrifuged at 10,000 *g* for 10 min. The top layer of 20 μL was injected into LC/MS. The plasma concentration of cefquinome was determined by an Agilent 1100 series LC/MSD (Agilent, Palo Alto, USA) system. Separation was achieved on Eclips plus C18 column (3.5 μm, 4.6 mm × 150 mm, Agilent, Wilmington, USA) with a guard column (4 mm × 3.0 mm, Phenomenex, Torrance, USA). The mobile phase consisted of 0.01% TFA in 10 mM ammonium acetate (A) and ACN (B) using a gradient elution as follows: 0-2 min, hold at 10% B; 2-6 min, liner gradient to 33% B; 6-9 min, liner gradient to 100% B; and 9-11 min, hold at 100% B at a flow rate of 0.6 mL/min. The mass spectrometer was run in the positive mode and selective ion monitoring mode focused on cefquinome at *m/z* 529.2 and ceftiofur (IS) at *m/z* 524.2.

Method validation

The validation scheme involved the analysis of calibration curves and quality control (QC) samples at different concentrations to determine linearity, inter- and intra-assay precision and accuracy, limit of quantitation according to the Korea Animal, Plant and Fisheries Quarantine and Inspection Agency (KQIA) guidance (9).

Calibration standards and QC samples during validation and pharmacokinetic study were prepared by spiking 90 μL of drug free plasma with 10 μL of standard working solutions of cefquinome. The calibration standards at seven levels over the concentration range from 0.02 to 5.0 μg/mL were extracted and assayed by the above-mentioned method. The calibration curves were constructed by the plots of the peak-area ratios of cefquinome to IS (y) versus the concentrations of the calibration standards (x). The accuracy and the precision of the assays for intra-day and inter-day determinations were evaluated by the analysis of QC samples at the concentrations of 0.02, 1.0 and 5.0 μg/mL (n = 4 for each concentration) on the same day and on 4 different days, respectively.

Pharmacokinetic evaluations and statistical analysis

Non-compartmental pharmacokinetic analysis was conducted using the software BA Calc 2007 (Kyunghee University, Seoul, Korea). The time of peak concentration (t_{max}) and the peak plasma concentration (C_{max}) after i.m. injection were determined from direct observation of plasma concentration vs. time curve. The terminal elimination rate constant (λz) was determined by the linear regression of the terminal portion of the log concentration-time profile. The elimination half-life ($t_{1/2\lambda z}$) was calculated as $0.693/\lambda z$. Area under the plasma concentration-time curve (AUC) was determined by the trapezoidal rule.

Bioequivalence of two cefquinome products was evaluated by the K-BE test 2002 program (Kyunghee University, Seoul, Korea). The two commercial cefquinome preparations were considered bioequivalent if the 90% confidence intervals (CI) for test/reference ratios of logarithmically transformed C_{max}, and $AUC_{0\rightarrow\infty}$ were within the range of 0.80-1.25 ($p < 0.05$).

Results and Discussion

The described analytical method was proven sensitive and accurate for determination of cefqinome plasma concentration. Comparison of the chromatograms of blank and spiked swine plasma samples indicated no significant interferences at the retention times of analytes and IS. The peaks of cefquinome and IS were observed at 7.7 and 8.4 min, respectively. The peak area ratios of cefquinome to IS were used for construction of the calibration curves, ranged from 0.02 to 5.0 μg/mL. The calibration curve was shown to have good linearity ($r^2 > 0.99$) over the range of 0.02-5.0 μg/mL. The mean precision values were less than 9.7% and accuracy values ranged from 93.5 to 99.8% for four replicates of quality control samples at the concentration levels of 0.02, 1.0, and 5.0 μg/mL. Inter-assay precision values were less than 13.2% and accuracy values ranged from 91.9 to 108.3% for QC samples at the same concentration.

The extent of absorption is the main characteristic of drug formulation, and therefore AUC is an important parameter for comparative bioequivalence study (6). However, the other two parameters, C_{max} and t_{max}, are also important features of the plasma level profile and could affect the therapeutic use of a drug (15).

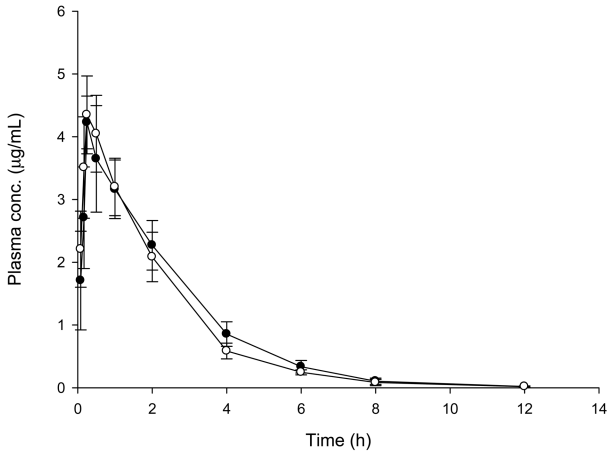

Fig 1. Plasma cefquinome concentration-time profiles of of two commercial cefquinome injectable solutions (test product, Cequus®, ○; reference product, Cobactan®, ●) following intramuscular injection at 2 mg/kg in 12 healthy piglets.

Table 1. Main pharmacokinetic parameters of cefquinome injectable products in 12 healthy piglets

Pharmacokinetic parameters (Unit)	Cequus® (test)	Cobactan® (reference)
C_{max} (μg/ml)	4.34 ± 0.58	4.22 ± 0.47
t_{max} (h)	0.38 ± 0.12	0.35 ± 0.13
$t_{1/2\lambda z}$ (h)	1.65 ± 0.28	1.66 ± 0.31
$AUC_{0\rightarrow 12}$ (h·μg/ml)	10.36 ± 1.94	10.18 ± 2.96
$AUC_{0\rightarrow\infty}$ (h· μg/ml)	10.43 ± 1.96	10.25 ± 2.98

C_{max}, peak plasma concentration; t_{max}, time of peak concentration; $t_{1/2\lambda}$, terminal half-life; $AUC_{0\rightarrow 12}$, area under the concentration-time curve from 0 to 12; $AUC_{0\rightarrow\infty}$, area under the concentration-time curve from 0 to infinity.

There were no side effects such as nausea, swelling and hardness at the injection site and severe pains, associated with the i.m. injection of two commercial products in piglets. The plasma concentration-time profiles and pharmacokinetic parameters after a single i.m. injection of 2 mg/kg of Cequus® (test product) or Cobactan® (reference product) in piglets are summarized in Fig 1 and Table 1, respectively. The mean plasma profile of Cequus® was very similar to that of Cobactan®. Both drugs were declined gradually over a period of 12 hours after the time of peak plasma concentrations. Following i.m. injection of Cequus® or Cobactan®, the t_{max} and $t_{1/2\lambda z}$ values were 0.38 ± 0.38 h vs. 0.35 ± 0.13 h and 1.65 ± 0.28 h vs. 1.66 ± 0.31 h, respectively. It is confirmed that the drugs are absorbed and eliminated relatively rapidly in piglets which is consistent with ducks, pigs and sheep (11,14,16). The mean peak levels in plasma (C_{max}) were 4.34 ± 0.58 and 4.22 ± 0.47 μg/mL, respectively, which is similar with wild boars (12). The $AUC_{0-\infty}$ of Cequus® (10.43 ± 1.96 h·μg/ml) was slightly higher than that of Cobactan® (10.25 ± 2.98 h·μg/ml), but there were no statistically significant difference. Bioequivalence between two commercial cefqunome products was determined by calculating 90% confidence intervals (90% CI) for the ratios of C_{max} and $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data, as shown in Table 2. There was no statistically significant difference between the test and reference drugs in the logarithmically transformed $AUC_{0-\infty}$ as well as logarithmically transformed C_{max} values, using analysis of variance (ANOVA). The 90% confidence interval for the ratio of the logarithmically transformed $AUC_{0-\infty}$ values of Cequus® over those of Cobactan® lay between 0.93 and 1.17, while C_{max} values lay between 0.94 and 1.12. Plasma levels may be used as surrogate parameters for clinical activity, therefore results of this study suggest equal clinical efficacy of the two commercial injectable formulation cefquinome.

Table 2. Comparison of 90% confidence interval for the log-transformed ratios of pharmacokinetic parameters of two commercial cefquinome injectable solutions (test product, Cequus®; reference product, Cobactan®) following intramuscular injection at 2 mg/kg in 12 healthy piglets

Pharmacokinetic parameters (Unit)	Geometric mean ratio (test/reference)*	90% confidence interval
C_{max} (μg/ml)	1.024	0.941-1.115
$AUC_{0\rightarrow\infty}$ (h·μg/ml)	1.042	0.927-1.172

*: ratios of logarithmically transformed

Conclusion

Statistical comparison of the $AUC_{0\rightarrow t}$, $AUC_{0-\infty}$ and C_{max} clearly indicated no significant difference between test product, Cequus®, and reference product, Cobactan®. The 90% confidence intervals for the ratio of C_{max} (0.94-1.12) and $AUC_{0-\infty}$ (0.93-1.17) values for the test and reference products were entirely within the KQIA acceptance range (8). Based on the above results, Cequus® is bioequivalent to Cobactan® in its extent of drug absorption. Therefore, it can be assumed that the two formulations are therapeutically equivalent and interchangeable in veterinary clinical practice.

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돼지에서 두 가지 Cefquinome 2.5% 제제의 생물학적 동등성 평가

송인배 · 김태원 · 이홍기 · 김명석* · 황윤환 · 박병권 · 임종환***† · 윤효인**[1]

충남대학교 수의과대학, *전라남도한방산업진흥원, **한국한의학연구소, ***B&C 바이오팜

요 약 : Cefquinome은 제4세대 cephalosporin으로 동물전용의약품으로 개발되었으며, β-lactamases에 대해서 매우 안정하고, 그람 음성 세균 및 양성 세균에 대한 광범위한 살균력을 가지고 있다. 본 연구는 현재 시판중인 cefquinome 주사제를 이유 자돈에 2 mg/kg 용량으로 근육 주사한 후 약물동태학적 특성을 파악하여 두 제제의 생물학적 동등성을 평가하였다. Cefquinome의 혈중 농도는 액체크로마토그래프/질량분석기를 이용하여 분석하였으며, 생물학적 동등성을 판정하기 위한 약물동태학적 인자로는 혈중 최고 농도 (C_{max})와 혈장 농도 곡선하 면적 ($AUC_{0\rightarrow\infty}$)을 사용하였다. 시험약과 대조약의 혈중 최고 농도는 4.34 ± 0.58 μg/mL과 4.22 ± 0.47 μg/mL로 각각 나타났으며, 혈장 농도 곡선 하 면적은 10.43 ± 1.96 μg·h/mL과 10.25 ± 2.98 μg·h/mL로 관찰되었다. 로그변환한 약물동태학적 인자의 평균비율의 90% 신뢰 구간은 C_{max}의 경우 0.941-1.115이었고, $AUC_{0\rightarrow\infty}$의 경우 0.927-1.172로서 생물학적 동등성 기준인 0.8-1.25를 모두 만족시켰다. 이상의 결과로 시판중인 두 cefquinome 제제는 생물학적으로 동등하다고 판단된다.

주요어 : 생물학적동등성, cefquinome, 액체크라마토그래프/질량분석기, 약물동태학