Comparative Pharmacokinetics and Bioequivalence Studies of Three Oral Cephalexin Monohydrate Formulations

Sumia S. Mohamed[1]✉, Mohamed A. Mustafa[2], Eltahir A. Ahmed[1], Nemat A. Algarai[1], Zoheir A. Alawad[1] and Abdulazim A. Ali[3]

[1] Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum, Sudan.
[2] Department of Medicine, Khartoum Teaching Hospital, Khartoum, Sudan.
[3] Department of Pharmaceutics, College of Pharmacy and Health Sciences, Ajman University, Ajman, UAE.

ABSTRACT

This paper aims at assessing the pharmacokinetics and bioequivalence of two locally manufactured cephalexin capsules in comparison with a reference product in Sudan. Bioequivalence of two locally produced cephalexin capsule formulations was investigated in 24 healthy volunteers following administration of a single dose (500 mg) of each product and a reference product in open randomized crossover studies within a one week washout period. Blood samples were drawn over a 6-hour interval and plasma concentrations of cephalexin were measured using HPLC-UV technique. Pharmacokinetic parameters and bioavailability measures were determined using non-compartmental analysis. Two one-sided t-test and ANOVA analysis showed no statistically significant differences in pharmacokinetic parameters between the two formulations and the reference product ($P > 0.05$). The mean values of AUC_{∞}, and C_{max}, are 33.4, 35 and 32.5 µgh/ml; 19, 17.4 and 17.3 µg/ml for reference product A and test brands B and C, respectively. T_{max} was about 1 hour for all three formulations. 90% Confidence Intervals (CI) of the ratios of means for the two formulations were 93.46 - 106.54 and 86.62 - 113.38% for AUC_{∞}, 86.65 - 113.35 and 88.25 - 111.75% for C_{max}, and 92 -107.9 and 89.9 – 110% for T_{max}. Since the 90% CI values were within the interval proposed by the FDA (80 – 120%), the test products are deemed bioequivalent to the reference product. The three formulations are expected to give the same levels and the same therapeutic effect and therefore can be used interchangeably.

Keywords: Cephalexin Capsules, Bioequivalence, Pharmacokinetics, Bioavailability.

INTRODUCTION

Cephalexin (7-(D-α-Amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid monohydrate), a semi-synthetic first generation cephalosporin antibiotic, is extensively used as antimicrobial agent in the treatment of Urinary Tract Infections (UTI), otitis media, skin and soft tissue infections and upper respiratory tract infection. While its use has largely been superseded by further generations of cephalosporins, it nevertheless remains an important antibiotic in this country. Cephalexin clinical efficacy, bioequivalence, pharmacokinetic profile and influence of coadministration of certain drugs were described in several reports.[1-13] In addition to its routine use, studies showed that it is effective in the treatment of UTI during pregnancy and is safe to the fetus and the mother[1] and UTI caused by Escherichia coli[2]. The efficacy of different doses of oral cephalexin in the treatment of uncomplicated skin infections was described in comparison with topical preparations and other oral cephalosporins.[3-5] A study found that cephalexin dosed

Received on 10/4/2010 and Accepted for Publication on 11/7/2010.
✉ E-mail: msumia@hotmail.com

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twice daily or three times daily and cephadroxil once daily appear equivalent in bacteriologic and clinical cure of Group A Beta Hemolytic *Streptococcal* (GABHS) tonsillopharyngitis.[6] Bioequivalence studies of different brands of cephalexin have been assessed in urine and plasma data from experimental animals and humans.[7-12] A randomized crossover study to investigate the influence of ranitidine or omeprazole on the pharmacokinetics and pharmacodynamics of cephalexin monohydrate showed that there were no significant pharmacokinetic interactions between cephalexin and ranitidine or omeprazole.[13]

Antibiotics represent about 40 % of the total market of drugs in Sudan. The local pharmaceutical industries (currently 23) cover approximately 36 % of the total antibiotic consumption. There are 39 registered brands of cephalexin (19 suspensions and 20 capsules), produced by 14 manufacturers from 9 countries of origin, five of which are Sudanese.[14] Substitution of one formulation for another has become a common clinical practice. Regulations for registration of products from the local industry are relaxed with respect to bioequivalence studies. Treatment failure with antibiotics is always attributed to their irrational use and/or resistance of microorganisms. The question remains, whether this is true or the failure is merely a bioequivalence problem. Therefore, it is important to investigate the disposition and bioavailability of antibiotic brands, especially those manufactured locally to ensure their bioequivalence to innovators products.

GENERAL EXPIREMENTAL

Subject: Twenty four healthy male Sudanese volunteers from the same ethnic group were recruited for the study through local advertisement. Their average age and weight were 24.0 ± 5.03 years (range 20 – 38 years) and 53.9 ± 8.49 Kg (range 44 – 70 Kg), respectively. All volunteers were subjected to medical history review and clinical laboratory tests for liver and kidney functions and complete haemograms before participation in the study. Those with abnormal biochemical and hematological tests were excluded from the study. None of the volunteers had a history of hypersensitivity to cephalosporins, penicillins, or other medications and were non smokers. They were asked to abstain from medicines, especially cephalosporin products two weeks prior to and during the trials. The volunteers were also asked to start fasting at midnight before the administration of cephalexin. The volunteers were given a full explanation of the purpose of the study and the extent to which it is maintained. Their agreements were recorded in written consent forms after the approval of the study by the Ethics Committee of the National Health Laboratory (ECNHL), Khartoum, Sudan.

Pharmaceutical Formulations: Reference formulation A: Keflex® capsules, product of Eli Lilly Italia S.P.A. Sesto Fiorentino-Florence, Italy, Lot No. S220319. Test formulation B: Amilexin® capsules, product of Amipharma Laboratories Ltd. Sudan, Batch No. CPF01. Test formulation C: Sigmacef® capsules, Sigma Tau SUDAN Ltd Drug Store, Sudan, Batch No. Ts 029-00.

Clinical Protocol: The study was conducted using an open randomized single dose crossover design. The subjects were admitted to the outpatient area of Khartoum Teaching Hospital. An intravenous heparin lock was placed in a suitable antecubital vein for blood sampling and patency was maintained with normal saline flushes. Single oral doses (500 mg) of each product were given to the subjects with a 250 ml glass of water. No food and/or drink, except water, were allowed following drug administration. Blood samples (2 ml) were collected in heparinized vacutainers at 0, 0.25, 0.5, 0.75,1, 1.5, 2, 3, 4, 5, and 6 hours after drug administration. A standard breakfast was served 3 hours after drug administration. At the end of the 6-hour sampling period, the lock was removed and a regular meal was provided. The patients were observed for a short period and discharged. A wash out period of one week was ensured between trials. The samples were centrifuged immediately and plasma was separated and stored at – 20 ^{o}C until analysis.

Analytical Method: The analytical procedure for determination of cephalexin in plasma was modified from the methods of Yost and Derendorf (1985)[15]. The reversed phase High Performance Liquid Chromatography (HPLC) system consisted of a solvent delivery pump (Perken Elmer, USA) equipped with manual injector (Rhyodine, USA), which was provided with 50 µl sample loop, a UV/VIS Spectrophotometeric detector (Lc 290 Thermoseparation, USA) set at 254 nm, integrator (Chrom jet, thermophenigan, USA). A C_{18} reversed phase column (3.9 x 3.125 mm, Water, USA) with a guard column was used for separation. Two hundred µl of plasma samples were used. Plasma proteins were precipitated with 50µl of 2% perchloric acid. Cefotaxim (20 µl) was added as an internal standard. The mixture was vortexed for 30 s and centrifuged for 5 min at 4000 rpm. Twenty µl of clear supernatant was injected to the HPLC column. The mobile phase (acetonitrile in phosphate buffer pH 4.5; 8:92) was prepared daily, filtered through 0.45 m nylon filter (Whatman, Maidstone, England) and degassed before use. The follow rate was set at 0.75 ml/min. The separations were performed at ambient temperature. The HPLC method was validated for linearity, specification, accuracy, precision and stability. Reasonable retention times were achieved for cephalexin (6 minutes) and cefotaxim (13 minutes) with a total run time of 18 minutes. This allowed complete analysis of samples from one volunteer in addition to a calibration curve within one day.

Pharmacokinetic Analysis: Pharmacokinetic parameters were determined by non-compartmental analysis using Microsoft Office Excel® (2007). The area under the curve from 0 – 6 hours (AUC_{0-6h}) was calculated by trapezoidal rule and the area from time 0 to infinity ($AUC_{0-\infty}$) was obtained from $AUC_{0-6h} + C_6/Ke$, where C_6 is the last measurable concentration. The area under the first moment curve from time 0 – 6 h ($AUMC_{0-6}$) was determined by trapezoidal rule for C.t versus t curves. $AUMC_{0-\infty}$ was estimated from $AUMC_{0-6} + C_6t_6/Ke + C_6/Ke^2$. Mean Residence Time ($MRT_{0-\infty}$) was obtained by calculating the ratio of $AUMC_{0-\infty}/AUC_{0-\infty}$. Peak plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) were directly observed from the individual plasma concentration versus time curves. The elimination rate constant (Ke) and elimination half-life ($t_{½}$) were calculated by least squares linear regression of the terminal concentration decay phase. Clearance divided by bioavailability (Cl/F) was calculated by dividing dose by $AUC_{0-\infty}$. The volume of distribution divided by bioavailability (Vd/F) is obtained from clearance divided by elimination rate constant. C_{max} and T_{max} together with $AUC_{0-\infty}$ were used to describe the rate and extent of cephalexin bioavailability, respectively.

Statistical Analysis: Pharmacokinetic parameters, expressed as mean ± SD (coefficient of variation %CV), of test products B and C and reference product A were tested statistically using statistical package for social sciences; SPSS® (Version 10). Bioequivalence measures were determined by calculating and comparing the ratios and differences of C_{max}, T_{max}, AUC_6 and AUC_∞ of test and reference formulations. Two one-sided t-test and one way analysis of variance (ANOVA) were used to determine the significance of association at 90% confidence level.[16] Differences in values were considered statistically significant if $p \leq 0.05$. ± 20% decision rule was used to evaluate bioequivalence results. Test formulations are declared bioequivalent if the 90% CIs for ratios of mean C_{max}, AUC_6 and AUC_∞ are within the United States Food and Drug Administration (FDA) acceptable interval of 80 – 120%.

RESULTS and DISCUSSIOIN

The dose administered was the normal dose employed for skin and soft tissue infections. It was well tolerated by the volunteers, no adverse effects were observed during or after the study period.

Figure (1) shows the mean (± SEM) plasma concentrations of cephalexin observed following the administration of the three capsule formulations. Plasma levels of cephalexin were comparable and not significantly different from those previously reported in healthy volunteers following oral administration of

equivalent doses.[7-13] Table (1) summarizes pharmacokinetic parameters of cephalexin following the administration of products A, B and C. Cephalexin was rapidly and completely absorbed from the gastrointestinal tract with maximum plasma levels reached in about one hour for the three formulations (range 0.75 – 1.5 hours). The mean values (± SD) of C_{max} were 19.05 ± 4.95 (range 14.07 – 29.36 µg/ml), 17.36 ± 4.07 (range 13.31 – 27.35 µg/ml) and 17.31 ± 5.28 (range 11.13 – 26.24 µg/ml) for products A, B and C. $AUC_{6-\infty}$ was ≤ 5% of AUC_{∞} for all formulations; 3%, 5% and 4% for A, B, and C, respectively indicating the appropriateness of the analytical method limits of quantification. The extent of absorption appears to be similar in all products; AUC_{∞} was 33.35 ± 4.97 µgh/ml, 35.07 ± 7.35 µgh/ml and 32.46 ± 13.34 µgh/ml. Cephalexin was rapidly cleared off plasma; elimination was almost complete in 6 hours with elimination half lives of about one hour. Clearances and volumes of distribution of the three formulations were comparable among the volunteers. No statistically significant differences (P > 0.05), in all pharmacokinetic parameters of cephalexin, were observed between the locally manufactured capsules and the reference product.

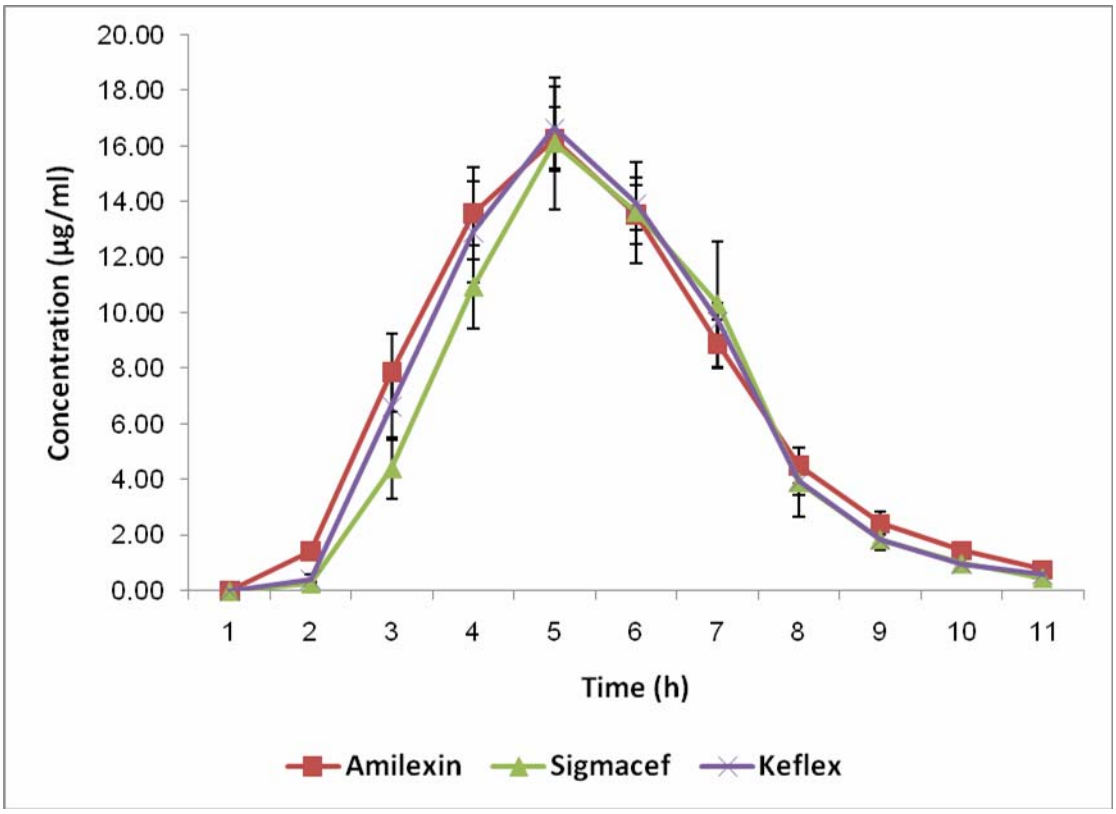

Figure 1: Mean ± SEM Plasma Concentration versus Time Following Oral Administration of a Single Dose of Amilexin®, Sigmacef and Keflex® Capsules to 24 Healthy Volunteers

Table 1: Pharmacokinetic Parameters (Mean ± SD (%CV)) Following Administration of a Single Dose of Reference and Test Formulations to 24 Healthy Volunteers.

Pharmacokinetic parameter	Reference A	Test B	Test C
T_{max} (h)	1.05 ± 0.26 (24.59)	1.10 ± 0.29 (26.68)	1.10 ± 0.29 (26.68)
C_{max} (µg/ml)	19.05 ± 4.95 (25.99)	17.36 ± 4.07 (23.43)	17.31 ± 5.28 (30.48)
AUC_{0-6h} (µgh/ml)	32.46 ± 4.18 (14.82)	33.35 ± 7.11 (21.32)	31.32 ± 12.47 (39.82)
AUC_{∞} (µgh/ml)	33.35 ± 4.97 (14.89)	35.07 ± 7.35 (20.95)	32.46 ± 13.34 (41.09)
$AUMC_{\infty}$ ($\mu g.h^2/ml$)	65.12 ± 17.35 (26.64)	72.46 ± 21.18 (29.23)	65.64 ± 32.35 (41.09)
MRT (h)	1.93 ± 0.35 (18.01)	2.04 ± 0.25 (12.08)	1.98 ± 0.32 (15.97)
ke (h^{-1})	0.72 ± 0.09 (12.71)	0.73 ± 0.15 (20.86)	0.73 ± 0.13 (18.19)
$t_{1/2}$ (h)	0.98 ± 0.13 (12.94)	0.99 ± 0.21 (21.37)	0.98 ± 0.16 (15.95)
CI/F (L/h)	15.34 ± 2.62 (17.08)	14.81 ± 2.98 (20.14)	17.81 ± 6.98 (39.16)
Vd/F (L)	21.68 ± 4.43 (20.45)	20.96 ± 5.06 (24.12)	24.64 ± 8.78 (35.65)

No statistically significant differences were observed among the three products (P > 0.05).

Statistical summary of comparative bioavailability data; least square geometric means, ratio of means and 90% Confidence Intervals (CI) for test products B and C are shown in table (2). The 90% CI of the ratios of mean for AUC_6, AUC_{∞}, C_{max} and T_{max} were 93.03 - 106.97 and 86.59 - 113.41%; 93.46 - 106.54, and 86.62 - 113.38%; 86.65 - 113.35 and 88.25 - 111.75%; and 93.40 - 106.60 and 91.55 - 108.45% for test formulation B and C, respectively. Since 90% CI gave differences within FDA bioequivalence acceptable range of 80%–120% for all bioequivalence measures, the three products can be considered bioequivalent.

Table 2: Statistical Summary of the Comparative Bioavailability Data: Least Square Geometric Means, Ratio of Means and 90% Confidence Intervals (%)

Parameter	Test B	Reference A	Ratio (%)	90% C.I.
AUC_{0-6h}	33.35	32.46	102.74	93.03 - 106.97
AUC_{∞}	35.07	33.35	105.15	93.46 - 106.54
C_{max}	17.36	19.05	91.27	86.65 - 113.35
T_{max}	1.10	1.05	104.76	93.40 - 106.60

Parameter	Test C	Reference A	Ratio (%)	90% C.I.
AUC_{0-6h}	31.32	32.46	96.49	86.59 - 113.41
AUC_{∞}	32.46	33.35	97.33	86.62 - 113.38
C_{max}	17.31	19.05	90.87	88.25 - 111.75
T_{max}	1.10	1.05	1.04.76	91.55 - 108.45

It can be concluded that since absorption profiles and disposition patterns of the locally produced test formulations are highly comparable with those of the standard product, they are expected to achieve the same levels and give the same clinical effect and can they be used interchangeably.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the volunteers and the technical staff at the Department of Pharmaceutics, for their support and encouragement throughout the preparation of this manuscript.

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مقارنة حركية الدواء و التكافؤ الحيوي لثلاثة من المستحضرات الفمية لعقار السفالكسين أحادي الماء

سمية سر الختم محمد[1]، محمد أحمد مصطفى [2]، الطاهر أحمد الطاهر[1] نعمات أحمد الفراي[1] ، زهير عبدالله العوض[1] و عبد العظيم أحمد علي[3]

[1] قسم الصيدلانيات، كلية الصيدلة، جامعة الخرطوم، السودان.
[2] قسم الأمراض الباطنية، مستشفى الخرطوم التعليمي، السودان.
[3] قسم الصيدلانيات، كلية الصيدلة، جامعة عجمان، الامارات العربية المتحدة.

ملخـص

تشمل هذه الورقة دراسة التمثيل الدوائي داخل الجسم لنوعين من كبسولات عقار السفالكسين المصنع محلياً. تم تقييم التمثيل الدوائي داخل الجسم لهذا العقار عن طريق تقييم الخصائص الحركية و مقياس التوافر الحيوي لمكون هذا العقار وهو السفالكسين أحادي الماء و مقارنتها بالمستحضر المرجعي الكيفلكس. أجريت هذه الدراسة في قسم الصيدلانيات – كلية الصيدلة – جامعة الخرطوم بمشاركة 24 من المتطوعين الأصحاء بعد تحقيق مقاييس التضمين و الاستثناء لهذه الدراسة. اتبعت التجارب الاكلينيكية طريقة الاختيار العشوائي في ثلاث مرات زمنية (ثلاث تجارب اكلينيكية). تم أخذ عينات الدم في أوقات محددة بعد أخذ جرعة واحدة من العقار (500 مج). تم قياس تراكيز السفالكسين في البلازما بواسطة التحليل الكروماتوغرافي (HPLC) باستعمال سفوتاكسيم كمادة قياسية داخلية للمقارنة. تم حساب حركية الدواء (المتوسط ± الانحراف المعياري) للسفالكسين بعد أخذ كبسولات الكيفلكس (أ) و كبسولات (ب) و كبسولات (ج) بالترتيب كالآتي: الزمن اللازم لملاحظة أعلى تركيز(T_{max}) 1.05 ±0.26 ساعة ، 1.10 ± 0.29 ساعة و 1.10 ± 0.29 ساعة. أعلى تركيز في البلازما (C_{max}) يساوي 19.05 ± 4.95 و 17.36 ± 4.07 و 17.31 ± 5.28 مايكروجرام/ مل. و المساحة تحت المنحني إلى ما لا نهاية (AUC_{∞}) تساوي 33.35 ± 4.97 و 35.05 ± 7.35 و 32.46 ± 13.34 مايكروجرام ساعة/مل و متوسط فترة بقاء الدواء داخل الجسم يساوي 1.93 ± 0.35 و 2.04 ± 0.25 و 1.98 ± 0.32 ساعة، و فترة نصف عمر الدواء تساوي 0.98 ± 0.13 و 0.99 ± 0.21 و 0.98 ± 0.16 ساعة. و قد أظهر التحليل الإحصائي أنه لا توجد فوارق ذات دلالات إحصائية بين كل من مستحضرات السفالكسين [كبسولات (ب) و كبسولات (ج)] و المستحضر القياسي كيفلكس/ كبسولات (أ). لمقارنة التوافر الحيوي النسبي لكبسولات (ب) و كبسولات (ج) مقارنة بالمستحضرالقياسي كيفلكس/ كبسولات فقد تم حساب مجال الثقة (90%) لنسبة متوسط كل من AUC_{∞} ، C_{max} ، T_{max} للمستحضرين (ب) و (ج) و قد كانت كالاتي: 93.46 – 106.54 و 86.62 – 113.38 %؛ 86.65 – 113.35 و 88.25 – 111.75 % ؛ 92 – 107.9 و 89.9 – 110 % . بما أن هذه النسب تقع في المجال المقترح من قبل منظمة الأغذية و الأدوية الأمريكية (80 – 120 %) فان المستحضرين تحت الاختبار يمكن اعتبارهما متكافئين حيوياً مع المستحضر المرجعي. خلصت الدراسة إلى أن التمثيل الدوائي داخل الجسم للمستحضرات المختبرة المصنعة محلياً يمكن مقارنته بصورة كبيرة مع التمثيل الدوائي داخل الجسم للمستحضر المرجعي. وعليه يمكن و صفها للعلاج بواسطة الأطباء بالتبادل.

الكلمات الدالة: كبسولات سيفالكسين، التكافؤ الحيوي، حركية الدواء، الصناعة المحلية.

تاريخ استلام البحث 2010/4/10 وتاريخ قبوله للنشر 2010/7/11.