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A randomized, single dose, two treatment, two period, crossover bioequivalence study of cefixime (as trihydrate) suspension in healthy participants comparing Acacime® suspension produced by ACAI / Iraq to Suprax® suspension produced by Hikma / Jordan

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Abstract:

Formulation of drugs affects their access into human body, thus bioequivalence studies are conducted to assess therapeutic equivalence between medicines which are produced by different manufacturers. In this study, bioequivalence of two cefixime formulations as powder for suspension, Acacime® manufactured by Arab Co. for Antibiotics Industries (ACAI) / Iraq, and Suprax®, manufactured by the Jordanian Co., Hikma, is tested. Twenty-four subjects had participated in the study which was designed as, a randomized, single dose, two period, crossover study. Cefixime concentrations in plasma were measured by validated bioanalytical method, using high-performance liquid chromatography with UV detection. The determined pharmacokinetics parameters were Cmax, AUC0–t and AUC0–∞ for cefixime. The mean results obtained for Acacime® and Suprax® were, for C max: 2.736 and 2.395 µg/ml, for AUC0–t: 16.787 and 16.579 µg/ml*h and, for AUC0–∞: 21.011 and 21.685 µg/ml*h, respectively. The 90% confidence intervals for AUC0-t, AUC0-∞ and Cmax were 92.3 – 110.8%, 84.8 – 102.7% and 96.3 – 117.9% respectively. This study revealed that both products were comparable in efficacy and safety, so they are considered as bioequivalent products and can be used interchangeably.

الخلاصة:

إن وضع المواد الدوائية في صيغ تركيبية و أشكال صيدلانية مختلفة يؤثر على وصولها إلى الجسم لذا فإن دراسات التكافؤ الحيوي يتم إجراءها لغرض المقارنة العلاجية بين المستحضرات المنتجة من قبل شركات مختلفة0 لقد تم في هذه الدراسة إختبار التكافؤ الحيوي لمادة سفكسيم بين مستحضري الشركة العربية للمضادات الحياتية (أكاي) و شركة الحكمة الأردنية حيث شارك اربع و عشرون شخصا في الدراسة التي صممت لتكون عشوائية ذات جرعة واحدة على فترتين بإسلوب التقاطع تم بعدها قياس تركيز سفكسيم في بلازما الدم بطريقة مثبتة عمليا بإستخدام منظومة الكروماتوغرافي السائل عالي الأداء مع الكشف بالإشعة فوق البنفسجية 0 كانت معايير حركية الدواء التي تم حسابها هي كل من التركيز الأعلى في الدم و المساحة تحت منحنى التركيز بمرور الزمن إلى اخر تركيز تم قياسه و إلى اللا نهاية0 تم الحصول على معدل القيم على النحو التالي لمستحضري شركة أكاي و شركة الحكمة على التوالي : التركيز الأعلى 2،736 و 2،395 مايكروغرام \ مل والمساحة تحت منحنى التركيز بمرور الزمن إلى اخر تركيز 16،787 و 16،579 مايكروغرام \ مل * ساعة و المساحة تحت المنحنى إلى اللا نهاية 21،011 و 21،685 مايكروغرام \ مل * ساعة 0 لقد أظهرت هذه الدراسة أن كلا المستحضرين متكافئين من حيث الوصول إلى الجسم و التحمل و بالتالي يمكن إعطاء إحدهما بديلا عن الاخر.

Introduction

Bioavailability and/or bioequivalence studies are required by health regulatory authorities since the generic products submitted for registration are imperative to be therapeutically equivalent to the innovator's products and clinically interchangeable[1]. In practice, bioequivalence studies are the most appropriate method for the demonstration of therapeutic

equivalence between medicinal products[2].

Pharmaceutical factors in the manufacture of drug formulation influence its bioavailability and it is important to detect any statistically significant clinical differences between the generic formulation, and the innovator (brand) product since different formulations between different manufacturers may lead to pharmacokinetics variations for the same drug. This does not imply that all pharmacokinetic parameters must be identical between the two products, but that any differences are not clinically significant, so that physicians are confident about the therapeutic outcome of the generic product with minimizing the cost of treatment[3].

Bioequivalence studies are conducted according to international ethical and scientific outlines. The studies should comply with Good Clinical Practice and Good Laboratory Practice[4]. Guidelines which adopt the basic principles for the studies were stated by regulatory authorities such as Food and Drugs Administration (FDA), The European Agency for Evaluation of Medicinal Products (EMEA) or World Health Organization (WHO)[5].

Cefixime is a third-generation cephalosporin antibacterial agent that acts by inhibiting synthesis of cell wall, with spectrum against many Gram-negative and few Gram-positive bacteria. The drug is used for treatment of susceptible infections including, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, in addition to gonorrhoea and urinary tract infections [6].

The objective of this study was to assess the bioequivalence of two oral suspension formulations for cefixime (as trihydrate) by comparing the rate and extent of absorption of the test product Acacime® produced by ACAI Co. / Iraq with that of innovator product Suprax® manufactured by Hikma / Jordan, following oral administration of 200 mg single dose to healthy, adult, male participants under fasting conditions. This assessment was through calculating the primary pharmacokinetics parameters, including the maximum plasma concentration (Cmax) which represents the absorption rate of drug, and the area under plasma concentration versus time curve from zero to the last measurable concentration (AUC0-t) and to infinity (AUC0-∞), which represent the extent or amount of drug that reach systemic circulation[7].

Materials

I.V cannula (Suru, India), 10-ml disposable syringe (QG, Qatar), 2.5-ml EDTA tubes (Sun, Jordan), 1.5-ml centrifuge tubes (eppendroff, Germany), cefixime trihydrate reference standard (USP, USA), ceftazidime working standard (LDP, Spain), perchloric acid (Fluka, Germany), sodium acetate (Scharlau, Spain), orthophosphoric acid (GCC, UK), potassium dihydrogen phosphate (BDH, Germany), acetonitrile (Biosolv, Netherlands).

Equipment

Analytical balance (Sartorius, Germany), pH meter (Inolab, Germany), deep freezer (Angeltoni, Italy), Centrifuge (Eppendrof, Germany), Vortex mixer (Stuart, UK), high performance liquid chromatography system (Knauer, Germany) with C18 column, 5µm, 250 × 4.6 mm, (Phenomenex, USA).

Clinical Study Design

The study was a comparative, open label, single dose, randomized, two treatments, two periods, two sequences, fasting, crossover bioequivalence study with 24 participants enrolled in to ensure adequate statistical results[8].

From the subjects underwent screening, 26 healthy subjects were selected by the clinical investigator as they met all the inclusion criteria which were age between 18 to 45 years, body mass index from 19 to 30 Kg/m2, wish to give written informed consent, clinical examination including vital signs and normal laboratory

results, assessed and accepted by the physician[9].

Participants should be adhered to some restrictions during the study periods including: No alcohol or xanthine-containing beverages e.g. tea, coffee and cola is consumed 48 hours prior to dosing and 12 hours after drug administration, smoking is prohibited during confinement, food and fluid intake was standardized for all participants in each study period, including a meal served 12 hours before dosing, followed by fasting till drug intake and continued 4 hours after which lunch was served. Water drinking was permitted 2 hours before dosing, in addition to 240 ml with the drug administration[10].

Randomization was done so that equal number of participants got either sequence T or R at each dosing period. Participants who had the T sequence, received the Acacime® in the first dosing period and Suprax® in the second dosing period, while participants who had the R sequence, received Suprax® in the first dosing period and Acacime® in the second dosing period. Seven days separated between the two periods[11].

Blood Sampling, collecting, and storage of samples

In each period, intravenous cannula was inserted into the upper limb of each participant and blood samples will be collected according to the following schedule: 8 ml pre-dose and 8 ml at 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 9.0 and 12.0 hours post dose. Samples collected into labeled EDTA tubes, then centrifuged at 3000 rpm for 5 min. and the separated plasma transferred into eppendroff labelled tubes to be stored frozen at – 60°C until analysis.

Drug Concentration Measurements

A validated bioanalytical method was developed for the determination of cefixime concentration in human plasma. Sample preparation started by spiking 900 µl of plasma sample with 60 µl of freshly prepared internal standard solution (200 µg /ml of ceftazidime) followed by the addition of 300 µl of 10% perchloric acid, mixed and centrifuged at 4000 rpm for 5 min., then 600 µl of the supernatant was neutralized with 300 µl of 1 M sodium acetate and 100 µl was injected into the chromatography system run in the following conditions: column C18, a mobile phase composed of phosphate Buffer pH 3 and acetonitrile (9 : 1) with UV detection at λ 280 nm and flow rate of 1 ml / min [12]. The calibration curves used were constructed in the range of 0.5 to 5.0 µg/ml. This method was shown to be valid for selectivity (no interference), linearity (R^2 mean > 0.99), accuracy (mean < 15.0%), precision (C.V < 15.0%) and stability (including short term stability at – 20 ºC for 7 days and three cycles of freeze / thaw stability)[13]. The results of analytical method validation are summarized in Tables 1,2 and 3.

Table-1: Regression equation parameters for the calibration curves used for determination of cefixime in plasma

#	Slope	Intercept	R^2
1	1.4677	0.1488	0.9968
2	1.4916	0.1133	0.9779
3	1.3527	0.1833	0.9876
4	1.3764	0.1550	0.9917
5	1.3630	0.1259	0.9946
Mean	**1.4103**	**0.1453**	**0.9900**

Table-2: Accuracy and precision of the method used for determination of cefixime concentration in plasma

Nominal concentration (µg/ml)	concentration (µg/ml)	%
Low calibration control 0.5 (µg/ml) (N = 6)	0.499	99.8
	0.398	95.0
	0.463	92.6
	0.499	99.8
	0.513	102.6
	0.535	107.0
Accuracy (%)	0.485	**99.4**
SD (+/-)	0.044	
Precision (%)		**9.07**
Medium calibration control 2.0 (µg/ml) (N = 6)	2.155	107.7
	2.187	109.3
	2.220	111.0
	2.076	103.8
	2.220	111.0
	2.220	111.0
Mean (µg/ml)	2.180	**109.0**
SD (+/-)	0.052	
CV (%)		**2.39**
High calibration control 4.5 (µg/ml) (N = 6)	4.22	93.7
	4.371	97.1
	4.515	100.3
	4.142	92.0
	4.414	98.0
	4.622	102.7
Mean (µg/ml)	4.381	**97.3**
SD (+/-)	0.163	

Table-3: Stability of cefixime at – 20 ºC for 7 days and 3 cycles of freeze / thaw in plasma

stability at – 20 ºC for 7 days		Freeze / thaw stability	
Relative concentration	%	Relative concentration	%
(Low) 2.2 µg / ml	97.6	**(Low)** 2.2 µg / ml	85.2
(High) 4.4 µg / ml	100	**(High)** 4.4 µg / ml	94.5
Mean	**98.80**	**Mean**	**89.85**

Results of Safety

All participants were monitored throughout the study for adverse events. They were asked specifically about any adverse event throughout confinement, in addition, sitting blood pressure and heart rate were

measured before dosing and at 1, 3 and 5 hours after dosing.

The result of clinical examination and vital signs measurement showed that both study medication products are well tolerated and did not induce any abnormal clinical findings during the conduction of study.

Results of Pharmacokinetics Parameters

Themean of cefixime concentrations versus time curves for all the 24 participants is shown in Figure 1. The pharmacokinetic parameters were derived from the curve of each participant. The primary parameters are themaximum plasma concentration (C_{max}), area under plasma concentration versus time curve from time zero to the last measurable concentration calculated by linear trapezoidal method (AUC_{0-t}) and area under the plasma concentration versus time curve from time zero to infinity ($AUC_{0-\infty}$), while the secondary parameters include time of the maximum plasma concentration (T_{max}), elimination rate constant (K_e) and the elimination half-life of drug during the terminal phase ($T_{1/2}$)[14]. These parameters were calculated using Kinetica® version 5 software and the results are shown in and figure 1.

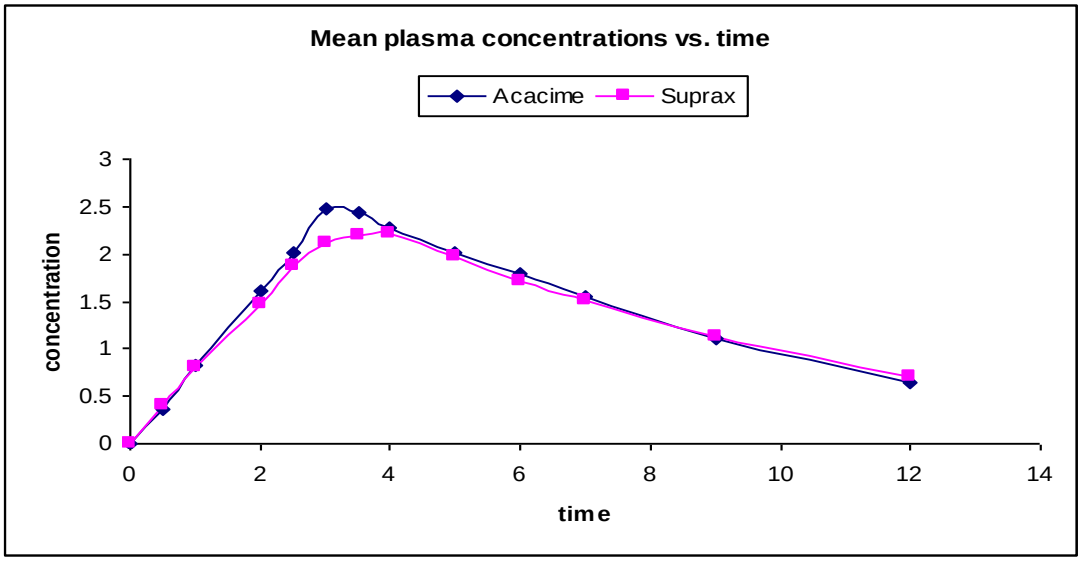

Figure-1:Mean cefixime concentrations versus time curve following single dose administration of Suprax® and Acacime® suspension

Table-4: Comparison between pharmacokinetic parameters following administration of Suprax® and Acacime® suspension

Subject No.	Innovator (Suprax®) product				Generic (Acacime®) product			
	T_{max}	**C_{max}**	**AUC_{0-t}**	**$AUC_{0-\infty}$**	**T_{max}**	**C_{max}**	**AUC_{0-t}**	**$AUC_{0-\infty}$**
1	3.5	2.450	16.386	26.765	4.0	2.593	13.362	19.107
2	4.0	2.920	19.969	22.728	4.0	2.51	14.764	18.592
3	3.0	2.859	15.818	21.651	5.0	2.463	14.145	20.356
4	4.0	2.655	17.078	22.872	4.0	1.953	14.391	20.988
5	4.0	3.080	21.088	25.874	3.0	3.576	19.706	25.266

6	3.5	1.845	14.274	17.565	3.0	2.543	14.022	17.355
7	4.0	3.354	24.706	29.579	3.5	3.195	17.213	20.289
8	4.0	3.244	26.374	36.234	3.5	5.057	29.604	35.113
9	3.5	1.695	12.499	16.467	3.0	1.422	9.246	10.901
10	4.0	1.707	13.699	19.126	3.0	1.755	10.673	14.188
11	3.5	3.001	20.248	24.109	4.0	2.088	16.488	21.690
12	3.0	2.408	15.549	17.813	3.5	3.02	17.668	22.427
13	2.5	2.475	15.318	21.623	3.0	2.393	13.636	17.592
14	3.0	1.685	9.734	11.389	3.5	3.457	20.115	24.103
15	4.0	3.082	24.069	31.339	3.5	4.415	29.559	37.329
16	3.0	2.534	19.259	27.941	3.5	2.332	17.457	19.678
17	2.5	2.025	15.116	21.388	3.5	2.940	18.674	23.754
18	3.5	2.215	15.546	20.290	3.0	2.725	18.169	20.245
19	3.5	2.351	13.913	15.920	3.5	2.703	18.171	21.162
20	3.5	2.192	16.340	22.723	3.0	2.703	14.675	16.629
21	3.0	1.402	9.044	11.298	3.0	2.224	12.846	15.064
22	4.0	2.327	14.498	17.747	3.0	2.565	14.399	15.886
23	4.0	1.825	12.699	20.428	3.0	2.480	17.239	24.849
24	3.0	2.150	14.671	17.580	3.5	2.551	16.669	21.700
Mean	3.48	2.395	16.579	21.685	3.44	2.736	16.787	21.011
S.D.	0.450	0.547	4.381	5.927	0.496	0.789	4.765	5.844

The acceptance range for bioequivalence is stated by most authorities as the 90% confidence interval for the primary parameters, so that the differences between generic and innovator for AUC and Cmax should lie within 80 - 125%, and may be extended to 75 - 130% for the highly variable drugs[15]. The point estimates and 90% confidence intervals for cefixime were calculated using Excel® software and the results were found within the acceptance range as summarized in table 5

Table-5: The point estimates and 90% confidence intervals for AUC0-t , AUC0-∞ , and Cmax following administration of Suprax® and Acacime® suspension

Pharmacokinetic parameter	Point Estimate	Lower Limit	Upper Limit
AUC_{0-t}	101.3%	92.3%	110.8%
$AUC_{0-\infty}$	96.9%	84.8%	102.7%
C_{max}	114.2%	96.3	117.9 %

Conclusion

In this study of cefixime for suspension, both formulation products were found comparable since the 90% confidence interval for the mean values of areas under the curves and peak plasma concentrations were within 80 - 125%, so the extent and the rate of absorption were comparable between the reference and the test formulation.

On the basis of pharmacokinetics and safety results, it can be concluded that the product Acacime® 100 mg / 5 ml suspension, manufactured by ACAI is

bioequivalent to Suprax® 100 mg / 5 ml suspension manufactured by Hikma.

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