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Bioequivalence and Pharmacokinetic Evaluation of Two Batches of Cephalexin Capsules in Healthy Volunteers

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Abstract

Aim: The aim of this study is to evaluate the difference in pharmacokinetic parameters between the same brand drugs containing Cephalexin as the active ingredient distributed to both the private sector and the governmental sector in Jordan.

Method: Plasma samples of two healthy volunteer groups have been examined at different time intervals after oral administration of Cephalexin 500 mg obtained from both private and governmental health sectors, and the pharmacokinetic parameters such as area under plasma concentration time curve (*AUC*), maximum plasma concentration (C_{max}), and time to reach maximum plasma concentration (T_{max}), have been measured after conducting high performance liquid chromatography (HPLC) experiment.

Results: Measurements have shown a significant difference in almost all parameters between Cephalexin from the private sector and that from the governmental sector that support the call for more control and regulations on drugs supplied to governmental health sectors.

Conclusion: More control on drugs supply for governmental hospitals and health centers and almost all drug companies must provide a proof for bioequivalence for their drugs going to that sector and not rely only on that bioequivalence comparison done for registration and FDA requirements.

Keywords: bioequivalence, cephalexin, pharmacokinetic, HPLC.

INTRODUCTION

Cephalexin a semisynthetic first-generation cephalosporin antibiotic having methyl and β-(2*R*)-2-amino-2-phenylacetamido groups at the 3- and 7- of the cephem skeleton, respectively [1]. It is one of the β-lactam antibiotics that are still one of the widely prescribed drugs for the treatment of wide range of infections, especially gram +ve origin such as throat, urinary tract, otitis media, skin and soft tissues, and upper respiratory tract infections [2]. It is from the first generation cephalosporin intended for an oral use and it is considered one of the safest antibacterial agents; in pregnant women it is among category B drugs and it is safe also to be given for breast-feeding moms [3].

Like other β-lactams, cephalexin exhibits time-dependent killing activity meaning that the time that concentrations in tissue and serum are above the minimum inhibitory concentration (T>MIC) is the PK/pharmacodynamic (PD) parameter that best correlates with efficacy [4]. The oral bioavailability of Cephalexin is almost 100% in the gastrointestinal tract [5]. Generally, total absorption of Cephalexin is not affected by food, although food might delay the absorption. Only 15% of Cephalexin binds to plasma proteins. Cephalexin is widely distributed in the body but does not enter the cerebrospinal fluid (CSF) in significant quantities. Cephalexin is not metabolized by liver enzyme and about 80% of dose is excreted unchanged in the urine [6].

Bioequivalence studies of different brands of Cephalexin have been assessed in urine and plasma data on experimental animals and humans in almost all the world; in world many of such studies have been conducted to compare different brands of cephalexin in terms of pharmacokinetic properties and extent of activity [7-8]. Several analytical procedures are available in the literature for the analysis of Cephalexin. These methods are spectrophotometry, high performance liquid chromatography (HPLC), Polarography and titrimetric analysis, and the reverse phase-HPLC [9-11].

Bioequivalence study is a common technique to assess and evaluate the comparison in pharmacokinetic properties especially on the rate and the extent of absorption for different generic formulation and compares that to the brand-name drug for the same active ingredient [12]. Many pharmacokinetic parameters have to be measured after administration of the same molar dose especially the area under the curve (*AUC*), the highest or maximum concentration that can be detected for drug (C_{max}) and the time needed for drug to each the maximum plasma concentration (t_{max}). If there is no significant different in these parameters, then both brand and generic formilations would be considered as bioequivalent, on the other hand, if a drug formulation differs in one of them, the Food and Drug Administration (FDA) would consider this drug as not equivalent to the brand product [13].

Generally, in bioequivalence studies the plasma concentration time curve is used to study the rate and the extent of absorption for both the generic and the originator drugs that having no significant difference in the *AUC*, C_{max} and T_{max}; that means that they are pharmaceutically equivalent and their bioavailability after administration lie within acceptable limits [14].

In this study, the experiment will not be conducted to compare a generic drug with the originator one having cephalexin as the active ingredient, it will be applied to study the compare the pharmacokinetic parameters of same Jordanian brand of Cephalexin but from two batches, one is normally sent to governmental health sectors and the other deposited in the private health hospitals and pharmacies. The need for such study came from the fact that there are many complications from patients claiming that drugs given to patients in hospitals from governmental sectors are not as effective as that purchasing from private pharmacies or hospitals. Therefore, the aim of our work here is to study whether there is a significant difference in pharmacokinetic properties and extent of absorption of 500 mg Cephalexin A capsule purchased from community pharmacy and that obtained from governmental hospital Cephalexin B. For non-bias results, the name of the company is not mentioned and we gave codes to the product.

MATERIALS AND METHODS

A comparative randomized, single dose, two-way crossover, open-label comparative pharmacokinetic study of Cephalexin (A) 500mg capsule distributed to private health sectors and Cephalexin (B) 500mg capsule distributed to governmental health sectors from the same Jordanian brand Cephalexin but from two batches.

A total 18 Jordanian healthy volunteers under fasting conditions was conducted on, their average age and weight were 22.0 ± 4.0 years (range 20-25 years) and 68.5± 9.5 Kg (range 70 – 95 Kg), respectively. Six of volunteers (group A) have taken Cephalexin (A) 500 mg and the other 6 volunteers (group B) have taken Cephalexin (B) 500 mg orally.

The HPLC system was comprised of a Shimadzu VP series pump (LC-10AT vp/FCV-10AL-vp, Kyoto, Japan) with solvent cabinet, auto-injector (SIL-10AD vp), UV/VIS detector (SPD-20AD vp) and computer software (VP-CLASS). Methanol was HPLC grade (May & Baker Ltd., Dagenham, U.K.). Cephalexin was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

Blood sample (7-8 ml) were drawn from vein by syringes in to heparinized blood tubes after 15, 30, 60, 90, 120, 240 minutes, then transferred immediately into polypropylene tubes and centrifuged within 5 min. at 500G for 15 min. 100µl of 5% per-chloric acid was added for each one milliliter of plasma. Cephalexin (100µl) was added as an internal standard. Cephalexin was extracted from human plasma samples by deproteinization using precipitation process. A 500µl aliquot form each plasma sample was transferred to a 5.0ml polypropylene tube. One millilitre of cold methanol was added. After slightly vortex mixing, the tubes were centrifuged for 15min. at 500G. A 100µl aliquot of the supernatant was transferred to the injection vials and 50µl were injected into chromatographic system. All samples from volunteers were analysed on the same day in order to avoid inter-assay variation. Plasma solutions were protected from the light and stored in a deep freezer at (2030 K) until getting analysed by reverse phase HPLC.

The Area under the curve (*AUC*) from time zero to the last measurable concentration (AUC0–t) was calculated using the trapezoidal rule. Maximum plasma concentration (C_{max}) and time to achieve maximum plasma concentration (T_{max}) were obtained directly from plasma concentration data. *AUC*, C_{max}, T_{max} obtained with the two preparations were analysed statistically using an analysis of variance (ANOVA) procedure, which distinguished effects due to subjects, periods, and treatment. The mean *AUC* and C_{max} values were calculated directly from the mean plasma concentration versus time for both groups. On the other hand, the T_{max} values of the two preparations were analysed using the Wilcoxon Signed Rank Test for paired samples. The decision rule was used to evaluate bioequivalence results and the test formulations are declared bioequivalence if the 90% CIs for ratios of mean C_{max} and *AUC* are within the United States Food and Drug Administration (FDA) [15] and it will be acceptable interval of values. A statistically significant difference was considered when $P < 0.05$.

RESULTS

The plasma concentration-time profiles for both groups were shown in Table 1 and Figure 4. It has been clearly observed that, Initially, plasma concentration was increased at the same rate with no significant variation between groups from time 0 to 30 minutes, p = 0. 507 as shown in Table 2 and Figure 1. Lag time was observed in four volunteers (volunteer No. 1 and 4 for group A and volunteer No. 1 and 3 for group B), the initial plasma concentration-time profiles for volunteers with lag time were not significantly different between groups as illustrated in Figure 2 and Figure 3).

The plasma concentration-time profiles from 60 to 240 minutes were significantly higher in group A, p <0.05. Maximum plasma concentration of group A was significantly higher comparing to group B according to declared bioequivalence of United States Food and Drug Administration (FDA) (CI >36.57%). T_{max} was not significantly varied between both groups (CI <2.5%). The area under plasma concentration-time curve was significantly higher in group A as observed by a statistical analysis of higher plasma concentration beginning after 30 minutes to the end of 240 minutes, p< 0.05, and by declared bioequivalence of United States Food and Drug Administration (FDA) (CI >30.98%), (Table 4).

Table 1: Plasma concentration-time profiles for Cephalexin A and Cephalexin B groups.

Cephalexin A plasma concentration µg/ml versus time							
Time (min)	Volunteer-1	Volunteer-2	Volunteer-3	Volunteer-4	Volunteer-5	Volunteer-6	Mean ± Standard deviation
15	0.724	0.000 BDL	1.194	0.000 BDL	5.418	4.261	2.899 ± 2.298
30	10.298	0.6	8.253	7.954	14.936	11.796	8.973 ± 4.836
60	9.602	1.992	5.699	10.667	8.314	8.501	7.463 ± 3.152
90	7.228	8.22	3.849	6.423	5.332	5.003	6.009 ± 1.593
120	4.318	8.742	2.448	5.1	3.906	3.481	4.666 ± 2.183
240	0.709	1.431	0.000 BDL	0.848	0.961	0.516	0.893 ± 0.344
Cephalexin B plasma concentration µg/ml versus time							
Time (min)	Volunteer-1	Volunteer-2	Volunteer-3	Volunteer-4	Volunteer-5	Volunteer-6	Mean ± Standard deviation
15	0.000 BDL	5.717	0.000 BDL	3.739	4.439	5.627	4.881 ± 0.958
30	0.681	4.034	6.658	8.357	6.957	8.737	5.904 ± 3.049
60	3.785	2.536	5.472	3.494	3.905	3.637	3.805 ± 0.951
90	4.497	1.557	3.854	1.777	3.078	2.130	2.816 ± 1.192
120	4.611	1.346	2.821	1.336	2.348	1.726	2.365 ± 1.246
240	0.876	0.04	0.157	0	0.162	0.072	0.261 ± 0.348

Table 2: Plasma concentration change from time 0 to 30 min for Cephalexin A and Cephalexin B in volunteers.

Volunteer	Plasma concentration at 30 min.		P-value
	Cephalexin A plasma concentration µg/ml	Cephalexin B plasma concentration µg/ml	
1	10.298	0.681	.507
2	0.600	4.034	
3	8.253	6.658	
4	7.954	8.357	
5	14.936	6.957	
6	11.796	8.737	

Table 3: Mean of plasma concentration-time profiles for Cephalexin A and Cephalexin B groups.

Time (minutes)	Cephalexin A mean plasma concentration µg/ml versus time	Cephalexin B mean plasma concentration µg/ml versus time	P-value
	Mean ± SD	Mean ± SD	
15	2.899± 2.298	4.881 ± 0.958	.21794
30	8.973± 4.836	5.904 ± 3.049	. 637
60	7.463± 3.152	3.805 ± 0.951	.02152
90	6.009± 1.593	2.816 ± 1.192	.00281
120	4.666± 2.183	2.365 ± 1.246	.04878
240	0.893± 0.344	0.261 ± 0.348	.00888

Table 4: Comparison between C_{max}, AUC and T_{max} of Cephalexin A and Cephalexin B groups

Drug	AUC (µg/min in ml)	C_{max} (µg/ml)	T_{max} (min)
Cephalexin A	1037.302	10.782	36.664
Cephalexin B	715.867	6.839	35.845

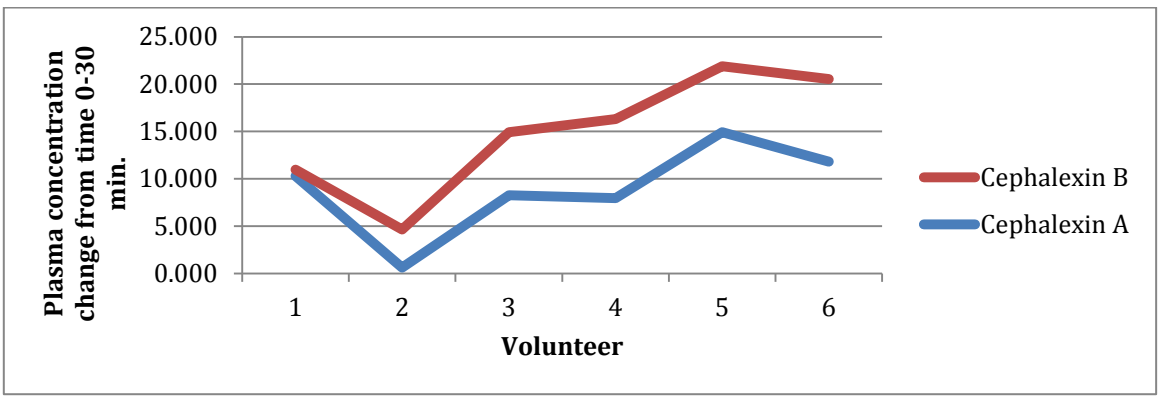

Fig. 1: Plasma concentration change from time 0 to 30 min for Cephalexin A and Cephalexin B in volunteers.

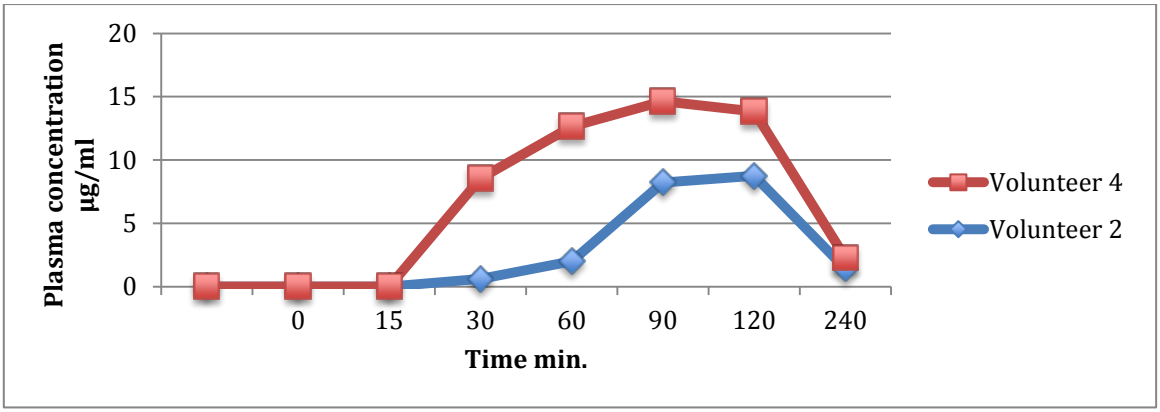

Fig. 2: Lag time for Cephalexin A

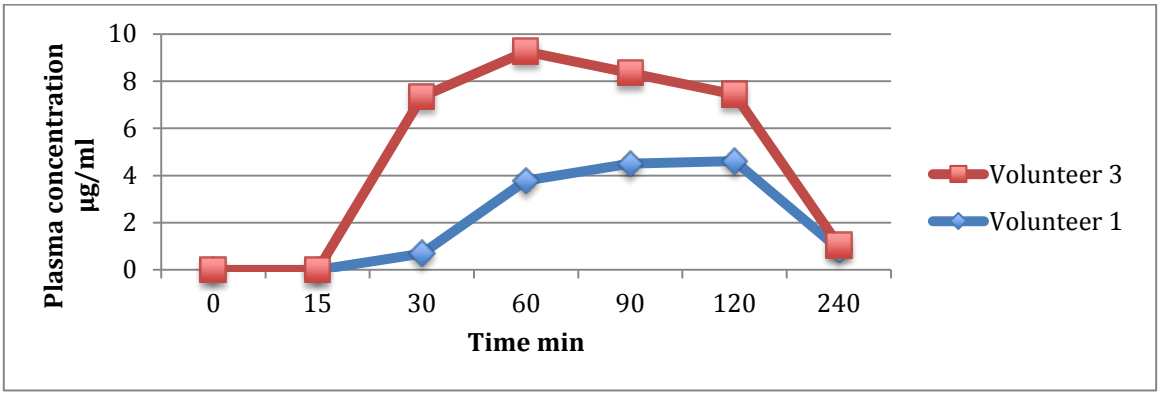

Fig. 3: Lag time for Cephalexin B

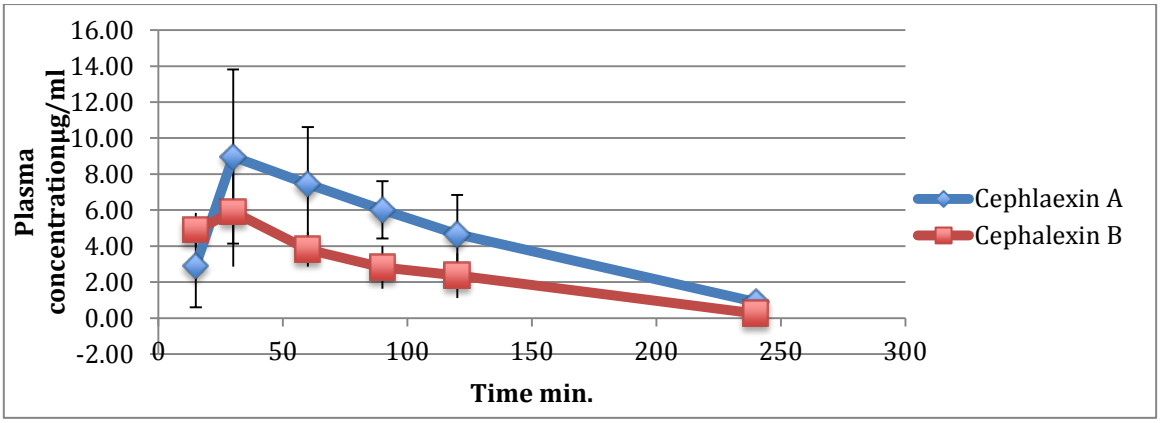

Fig. 4: Mean plasma concentration-time profiles for Cephalexin A and Cephalexin B groups.

DISCUSSION

Two products are considered to be bioequivalent when the rate and extent to which the active ingredient or therapeutic ingredient is absorbed and become available at the site of drug action [16]. Bioequivalence studies are used in a variety of situations, mostly to compare the pharmacokinetics of a generic version of an approved off-patent product [17]. Additionally, bioequivalence is widely used to compare the pharmacokinetic parameters between different batches of drug product from the same manufacturing company [18].

In this study, volunteers under taking Cephalexin A has significantly higher C_{max} and *AUC*, which means that Cephalexin A has much better oral bioavailability and extent of absorption compared to Cephalexin B group. Based on that, Cephalexin A which sent to private sectors and community pharmacies, has better drug profile in terms of extent of absorption, rate of absorption as well as duration of pharmacological action that strongly depends on how long the drug will stay in blood circulation in its effective concentration.

The significantly higher C_{max} observed with Cephalexin (A) could be result from higher rate and/or extent of absorption, whereas, the larger AUC is rate independent, and mainly depend on the extent of drug absorption [19].

Volunteers under taking Cephalexin A has significantly higher C_{max} and *AUC*, which means that Cephalexin A has much better oral bioavailability and extent of absorption compared to Cephalexin B group. Based on that, Cephalexin A which sent to private sectors and

community pharmacies, has better drug profile in terms of extent of absorption, rate of absorption as well as duration of pharmacological action that strongly depends on how long the drug will stay in blood circulation in its effective concentration. Moreover, the fact that the C_{max} is higher in Cephalexin A than Cephalexin B group (Table-4) reflects that the rate of absorption in this group is higher the rate of elimination which could be as a result of either lower cephalexin content in Cephalexin B, difference in formulation excipients and their relative concentrations or might be due to undiscovered health problems or physiological and biochemical variations between volunteers.

Deep insight to the plasma concentration vs time curve for both groups demonstrated the significant difference in almost all Pharmacokinetic parameters especially the C_{max} and AUC that strongly recommend more study on the factors that were behind these results although these were due to defect on the formulation, patient variations or formulation differences

CONCLUSION

The current work has focused on a bioequivalence study comparing two batches of Cephalexin Jordanian brand to measure the pharmacokinetic differences between drugs sold to governmental sectors and that sold to private sector. Results have shown significant difference especially in the C_{max} and *AUC* that justified the feedback obtained from patients visiting governmental hospitals about the ineffectiveness and delayed activity of antibiotics purchased from that hospitals compared to drugs obtained from private ones. Our work supports the call for more control on drugs supply for governmental hospitals and health centers and almost all drug companies must provide a proof for bioequivalence for their drugs going to that sector and not rely only on that bioequivalence comparison done for registration and FDA requirements

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Authors' Contribution

All authors have contributed to this study

Conflict Of Interest

Authors have no conflict of interest.

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