Research Article

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The Bioequivalence Study of Two Cefdinir 250 mg/5 mL Oral Suspension Formulations in Healthy Males Under Fasting Conditions

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ABSTRACT

A new liquid oral formulation of cefdinir has been developed and a bioequivalence study was conducted. This single-center study was designed as an open-label, randomized, two-period, cross-over trial, and was performed with healthy males under fasting conditions in compliance with Good Clinical Practice (GCP) principles. Two 250 mg/5mL suspension formulation of cefdinir was compared in terms of their pharmacokinetic properties and the bioequivalence of the new formulation was assessed according to the requirement of the authorities. The blood samples of the volunteers were taken at certain points specified to cefdinir, to evaluate the plasma concentrations and pharmacokinetic properties of two cefdinir formulations by using a validated LC-MS/MS analytical method. The bioequivalence of the new formulation has been shown and the tolerability of both products was acceptable.

Keywords: bioequivalence, cefdinir, cephalosporin, GCP, LC-MS/MS

1. INTRODUCTION

A semisynthetic cephalosporin that is used for the treatment of mild to moderate infections, cefdinir, is used for a wide-ranging spectrum of gram-negative and gram-positive bacterial strains [1,2]. While the solid formulations are prescribed to adults, the liquid formulations are generally preferred in pediatric patients in terms of dose adjustment and ease of drug administration. Besides, cefdinir has been reported to be preferable to other antibiotics in the sense of taste and palatability among children [3,4] and the suspension formulation is generally prescribed as either 7 mg/kg twice a day or 14 mg/kg once a day of oral ingestion [5]. However, cefdinir exerts low oral bioavailability and dose-disproportionality. Previous studies reported that the estimated absolute bioavailability of the suspension is 25% [5].

For pediatric patients and adults who cannot orally administer solid dosage forms, a novel cefdinir 250 mg/5mL suspension formulation has been developed by Elixir İlaç Araştırma ve Geliştirme AŞ (Ankara, Turkey) for İ.E. Ulagay İlaç Sanayii Türk A.Ş. (Istanbul, Turkey). Together with this, to assess the bioequivalence of the new formulation to the reference product, Cefdinir 250 mg/5 mL for Oral Suspension, a comparative bioavailability study was conducted in healthy males by following the regulations of International Council on Harmonization (ICH), European Medicines Agency (EMA) and Food and Drug Administration (FDA).

2. MATERIALS AND METHODS

2.1. Clinical Study

This study was designed as a single dose, open-label, randomized, cross-over, two-period study, and 24 healthy adult males aged 18 – 55 years with a body mass index within 18.5 – 30 kg/m^2 were enrolled. The study was conducted at FARMAGEN Good Clinical Practice Center. Before enrollment, all volunteers willing to take part in the clinical study read, understood, and signed the written informed consent form on their own free will. Besides, they understood that they could withdraw from the study anytime without specifying any reason.

However, the participants who have the following conditions were excluded from the study: (*i*) who have atopic constitution or asthma and/or known allergy for cefdinir and/or other cephalosporin group antibiotics and/or penicillin or any excipients of the study products; (*ii*) who have any history of cardiovascular, neurological, musculoskeletal, hematological, hepatic, gastrointestinal, renal, pulmonary, endocrinological, metabolism disorders; (*iii*) the presence of clinical relevance with the above-mentioned conditions or having a history of malabsorption or other conditions that might affect the pharmacokinetics of study drugs; (*iv*) who have already been included in another clinical study or took long half-life medications, enzyme-inducing or organotoxic drugs within 4 weeks and/or depot formulations within 6 months before the trial starts.

Consumption of foods or drinks containing methylxanthines over a certain limit, and/or grapefruit or grapefruit juice during 7 days prior to drug administration and throughout the study, having a history of drug or alcohol abuse and/or having positive alcohol breath test and/or drug test are counted as grounds for exclusion.

Erciyes University Ethical Committee of Bioequivalence/Bioavailability Studies (2019/73; 22.05.2019) and Turkish Medicines and Medical Devices Agency (13.06.2019) reviewed and approved this study in compliance with Declaration of Helsinki and Good Clinical Principles (GCP) [6].

The test drug used was Sefdinir 250 mg/5 mL Oral Süspansiyon (İ.E. Ulagay İlaç Sanayii Türk A.Ş., Istanbul, Turkey) (Batch No: XAEH001A; Expiration Date: 03.2021); the reference drug used was Cefdinir 250 mg/5 mL for Oral Suspension (Sandoz, USA) (Batch No: HH4680; Expiration Date: 08.2019).

The clinical study period spanned approximately four weeks. Pre-study screening, 7 days of wash-out period, and final examination after the last blood sampling were also counted in the clinical period. Initial standard clinical and biochemical screenings were done on blood and urine samples of the volunteers to check their eligibility. Brief anamnestic and demographic data, physical examination, body temperature determination, weight, height, standard ECG (12 lead), blood pressure (BP), and pulse rate (PR) measurements were recorded together with all necessary biochemical tests (HBsAg, HCV-Ab and HIV-Ab tests included to avoid possible infections) which were carried out in a certified local laboratory. Urine drug screening and alcohol breath tests were also performed prior to the study.

On the day before the dosing day, enrolled participants were hospitalized, followed by the randomization at the GCP clinic. On hospitalization days, a standardized dinner was served during each period.

On dosing days of each of the two periods, pre-dose blood sampling was done just before the study product administration. Then, volunteers were administered with their study products, 5 mL of the test or reference cefdinir suspension after staying 10 h fasted. The volunteers who received the test product in the first period got reference product in the second period or vice versa. A standardized lunch was provided 4 h; and a standardized dinner was provided 10 h after dosing in each period. Blood samplings were done at the following time points: at 0.00, 0.33, 0.66, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 14.00 h and the samples were collected into polypropylene tubes using K_2EDTA as anti-coagulating agent. After sampling, the samples were immediately refrigerated at approximately 4 °C, for no more than 30 min. Following the centrifugation (3,000 rpm, 4 – 6 °C, 10 min), the separated plasma from each sample was transferred into two 3 mL transparent, polypropylene tubes, transferred to a deep freezer and stored at -70 °C until the clinical study ended. Later, they were transported to the bioanalytical center for the analyses.

2.2. Bioanalytical Study

Bioanalyses have been run using a validated chromatographic in-house method at Novagenix Bioanalytical R&D Center (Ankara, Turkey). In contrary to the clinical study, the bioanalytical studies were conducted as blinded to avoid bias for keeping compliance with GCP. Analytical reference standard of cefdinir was supplied from Covalent Laboratories Private Limited (India), and the internal standard (IS), cefaclor was supplied from Alsachim (France). Solvents; methanol, acetonitrile, dichloromethane, and formic acid were supplied from Merck (Germany). Ultrapure (Type 1) water was supplied from Millipore Milli-Q water purification system; K_2EDTA blank human plasma was supplied from Gaziantep University Farmagen GCP Centre (Turkey).

A Shimadzu Liquid Chromatograph Mass Spectrometer LCMS-8040 was used as the LC-MS/MS system. Atlantis dC18, 3 µm (4.6 × 75 mm) column was chosen with a mobile phase consisting of 0.2% formic acid and acetonitrile/methanol (1:1) (80/20, *v*/*v*) with a column oven temperature maintained at 30 °C. The flow rate was 0.9 mL/min. Electrospray ionization was performed in MRM mode to detect *m*/*z* 396.00 > 126.05 (cefdinir) and *m*/*z* 368.00 > 118.05 (cefaclor) ions, simultaneously. The total run time for the method was 5.5 min.

From 1 mg/mL stock standard solutions of cefdinir, working solutions in the concentration range of 0.4 – 180 µg/mL were prepared, while the working IS was prepared at a concentration of 10 µg/mL and was stored at -70 °C. Calibration standards were prepared by spiking the appropriate amounts of standard solutions into the blank plasma to obtain final concentration levels between 20 – 9,000 ng/mL; the quality control samples were prepared at concentrations between 20 – 6,750 ng/mL. The lower limit of quantification (LLOQ) was 20 ng/mL. Stock solutions of cefdinir, IS, calibration standards, and QC samples were stored at -70 °C freezer until analysis.

Protein precipitation followed by liquid-liquid extraction was selected to extract cefdinir and the sample preparation was done according to the bioanalytical center sample preparation SOPs.

The method was validated for selectivity, specificity, carry-over, linearity, precision and accuracy, recovery, dilution integrity, influence of hemolyzed and hyperlipidemic plasma, drug-drug interaction, matrix effect and stabilities, and the validation was performed with K_2EDTA human plasma according to EMA Guideline on Bioanalytical Method Validation [7]. As a summary of the validation, the developed method showed no interference, and no influence of hemolyzed or hyperlipidemic plasma were observed. There were no drug-drug interactions and carry-over effects, and calculated concentrations of the calibration curve fulfilled the acceptance criteria for linearity, precision and accuracy. Recovery, stability and matrix effect of the quality control samples were also met with the acceptance criteria described.

The analytical curves were constructed from a plasma sample processed without IS (blank), a plasma sample processed with IS (zero), and eight concentrations of cefdinir, including the LLOQ, ranging from 20 to 9,000 ng/mL. The concentrations were calculated using peak area ratios and the linearity of the calibration curve was determined using least squares regression analysis. The acceptance criteria for each calculated standard concentration were not more than 15% deviation from the nominal value,

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except for the LLOQ which was set at 20%. The within-batch precision and accuracy were evaluated by analyzing QC samples at different concentration levels between 20 – 6,750 ng/mL. The matrix effects were evaluated by comparing the peak areas of post-extraction blank plasma that were spiked at certain concentrations of QC samples with the areas obtained by the direct injection of the corresponding standard solutions.

In-house high-performance liquid chromatography with mass spectrometry method (LC-MS/MS) was developed and validated to quantify cefdinir in plasma. The plasma samples were maintained at -70 °C during the assay.

2.3. Pharmacokinetic and Statistical Analyses

In order to demonstrate bioequivalence with a power of 80% and a test/reference parameter ratio between 0.95 and 1.05, 24 volunteers were included and completed the study.

The maximum plasma concentration (C_{max}) and area under the curve from time 0 to the last sampling time ($AUC_{0\text{-}tlast}$) were considered as the primary target variables; area under the curve from time 0 to the infinite time ($AUC_{0\text{-}\infty}$), time to reach the peak concentration (t_{max}), terminal half-life ($t_{½}$), terminal disposition rate constant (λ_z) and mean residence time (*MRT*) were considered to be secondary target variables.

C_{max} and t_{max} for cefdinir were obtained directly by plasma concentration-time curves, while $AUC_{0\text{-}tlast}$ was calculated using the trapezoidal rule, and $AUC_{0\text{-}\infty}$ was calculated by summing $AUC_{0\text{-}tlast}$ and extrapolated area. The latter was determined by dividing the last measured concentration by λ_z which was estimated by regression of the terminal log-linear plasma concentration-time points.

C_{max} and $AUC_{0\text{-}tlast}$ were tested for statistically significant differences by means of the analysis of variance (ANOVA) test procedure after logarithmic transformation (ln). The effects of ANOVA were treatment, period, sequence, and volunteer within the sequence and tested at 5% level of significance. All statistical analyses were done using Phoenix WinNonlin (Version 8.1, Certara L.P.).

For the assessment of bioequivalence, 90% CI for the ratio of mean values of C_{max} and $AUC_{0\text{-}tlast}$ which are indicatives of the rate and extent of absorption, respectively, were calculated using ln-transformed data and the acceptance limits for bioequivalence criteria, 80.0 – 125.0%.

3. RESULTS AND DISCUSSION

To enroll in this study, 30 volunteers were screened; 24 of them were included and randomized into two groups. One participant left the study of his own will, and a replacement was done. 24 participants completed the clinical study. The mean ± SD age of volunteers was 23.58 ± 3.98 years and the mean ± SD body mass index (BMI) was 24.32 ± 3.27. The demographic data of volunteers are presented in Table 1. There was no protocol deviation through the clinical period.

The wash-out duration has been found sufficient since no pre-dose concentration of cefdinir was detected at t_0 samples of the second period. As primary variables, the mean ± SD of C_{max} were found 1,858.99 ± 651.21 ng/mL and 1,886.93 ± 521.45 ng/mL, and the mean ± SD of $AUC_{0\text{-}tlast}$ was found 9,143.81 ± 2,492.68 h.ng/mL and 9,310.32 ± 2,471.72 h.ng/mL for the test and reference products, respectively (Table 2).

In Table 2, the pharmacokinetic parameters of the study drugs; in Table 3, the geometric least square means, ratios, and 90% Confidence Intervals (CIs) are shown. The 90% CIs for the geometric mean ratios of C_{max} and $AUC_{0\text{-}tlast}$ have been found as 88.51 – 105.84% and 91.14 – 105.77%, respectively (Table 3).

Table 1. Demographic data of the volunteers.

n = 24	Age	Weight (kg)	Height (cm)	BMI
Mean	23.58	77.29	177.79	24.32
SD	3.98	12.34	6.86	3.27
Minimum	19	57	165	18.6
Maximum	37	103	195	30

Table 2. The arithmetic mean ± SD of the pharmacokinetic parameters of single oral dose of 250 mg cefdinir in test drug (Sefdinir 250 mg/5 mL Süspansiyon, İ.E. Ulagay İlaç Sanayii Türk A.Ş., Istanbul, Turkey); and the reference drug (Cefdinir 250 mg/5 mL for Oral Suspension, Sandoz, USA) in healthy adult male volunteers under fasting conditions (Arithmetic Mean ± SD) (n = 24).

Parameters (Units)	Test (T)	Reference (R)
C_{max} (ng/mL)	1858.99 ± 651.21	1886.93 ± 521.45
$AUC_{0\text{-}tlast}$ (ng.h/mL)	9143.81 ± 2492.68	9310.32 ± 2471.72
$AUC_{0\text{-}\infty}$ (ng.h/mL)	9258.54 ± 2486.56	9420.11 ± 2447.67
t_{max} (h)	3.17 ± 0.71	3.15 ± 0.89
$t_{½}$ (h)	1.64 ± 0.18	1.65 ± 0.15
λ_z (1/h)	0.43 ± 0.05	0.43 ± 0.04
MRT (h)	4.47 ± 0.60	4.31 ± 0.46

Table 3. Geometric Least Square Means, Ratio and 90% CIs of the test drug (Sefdinir 250 mg/5 mL Oral Süspansiyon, İ.E. Ulagay İlaç Sanayii Türk A.Ş., Istanbul, Turkey) and the reference drug (Cefdinir 250 mg/5 mL for Oral Suspension, Sandoz, USA) in healthy adult male volunteers under fasting conditions.

Parameter	Difference	DiffSE	TESTLSM	REFLSM	Ratio	90% CI	CV%	Power%
$\ln(C_{max})$	-0.0327	0.0521	7.4695	7.5022	0.9678	88.51 – 105.84	18.19	97.02
$\ln(AUC_{0\text{-}tlast})$	-0.0183	0.0433	9.0862	9.1045	0.9819	91.14 – 105.77	15.10	99.82
$\ln(AUC_{0\text{-}\infty})$	-0.0182	0.0414	9.0997	9.1179	0.9820	91.46 – 105.43	14.42	99.92
t_{max} (h)	0.0200	0.1336	3.1713	3.1513	1.0063	93.36 – 107.91		
$t_{½}$ (h)	-0.0131	0.0235	1.6363	1.6494	0.9920	96.75 – 101.65		
λ_z (1/h)	0.0049	0.0063	0.4283	0.4235	1.0115	98.61 – 103.69		
MRT (h)	0.1603	0.0756	4.4632	4.3029	1.0372	100.71 – 106.74		

The average plasma concentration-time curves and the average ln plasma concentration-time curves of study drugs are displayed in Figure 1 and 2, respectively.

As the secondary variables, the median of t_{max} for study drugs was found as 3 h and ranged from 2.0 to 5.0 h for the test, and from 1.66 to 6.0 h for the reference products. In addition, the mean ± SD of $t_{1/2}$ for the test and reference products were found 1.64 ± 0.18 h and 1.65 ± 0.15 h, respectively (Table 2).

There were no serious adverse reactions or adverse events reported throughout the study.

Cefdinir's broad spectrum against various strains gives rise to its value in the treatment of mild and moderate infections. Together with this, its liquid formulation makes cefdinir preferable among pediatric patients. Therefore, a generic formulation of cefdinir suspension has been developed for a more affordable option, and in order to be marketed, the bioequivalence of the formulation needs to be proven according to the regulations.

Statistical analyses showed that there is no statistically significant difference between the two formulations. Intrasubject variabilities were found as 18.19% and 15.10% for the primary variables.

4. CONCLUSION

Since the 90% CIs for the test/reference geometric mean ratios for C_{max} and $AUC_{0\text{-}tlast}$ of cefdinir are within the acceptance limits, it is concluded that the

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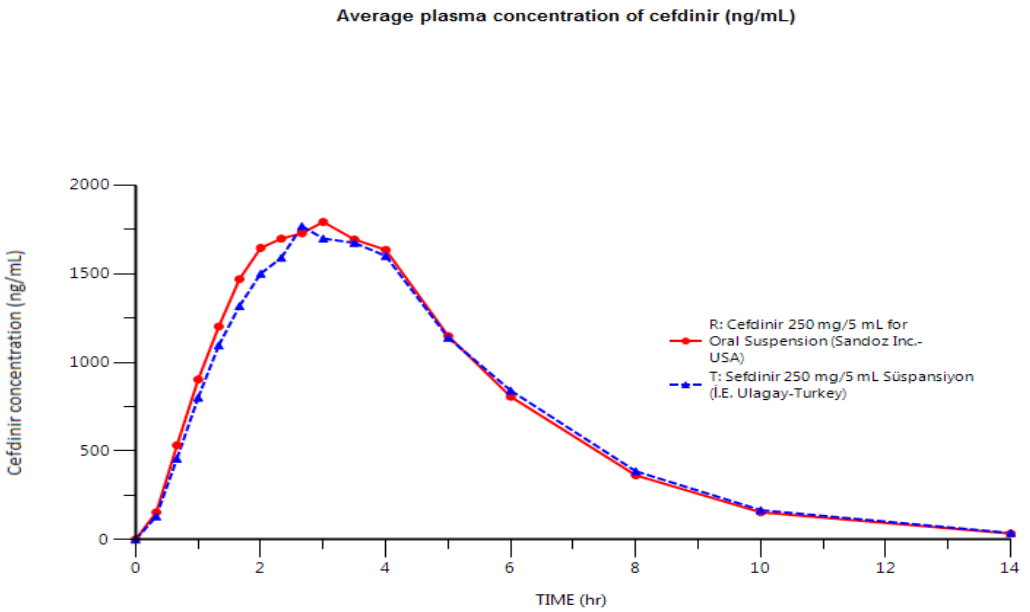

Fig. 1. Mean plasma concentration-time curves of cefdinir after a single dose of the test drug (Sefdinir 250 mg/5 mL Oral Süspansiyon, İ.E. Ulagay İlaç Sanayii Türk A.Ş., Istanbul, Turkey) and the reference drug (Cefdinir 250 mg/5 mL for Oral Suspension, Sandoz, USA) of oral cefdinir in healthy adult male volunteers (n = 24) under fasting conditions.

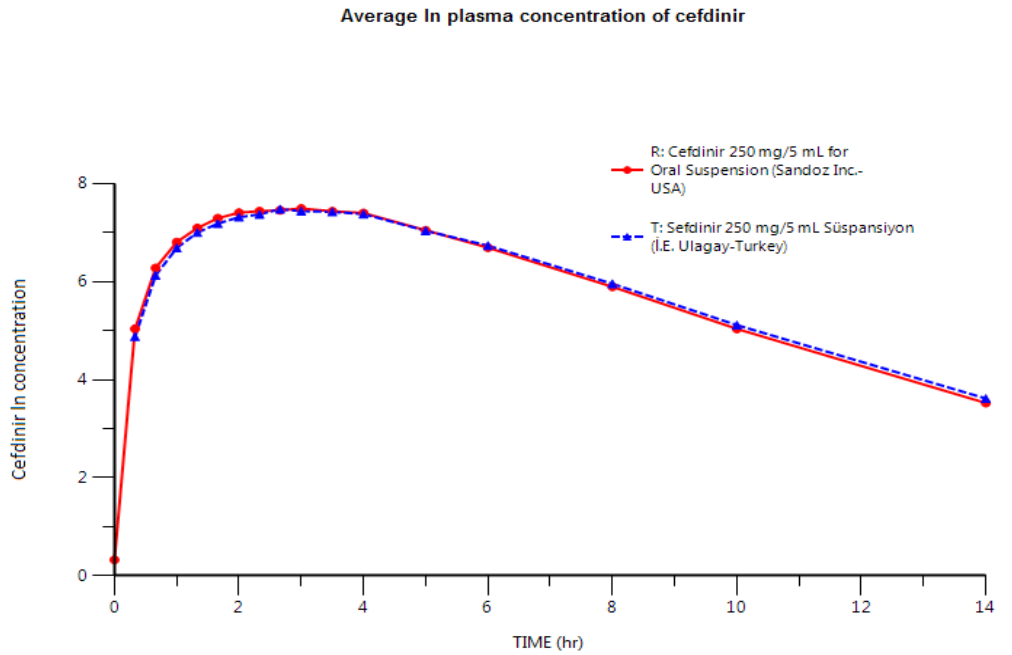

Fig. 2. Average ln plasma concentration curves of cefdinir after a single dose of the test drug (Sefdinir 250 mg/5 mL Oral Süspansiyon, İ.E. Ulagay İlaç Sanayii Türk A.Ş., Istanbul, Turkey) and the reference drug (Cefdinir 250 mg/5 mL for Oral Suspension, Sandoz, USA) of oral cefdinir in healthy adult male volunteers (n = 24) under fasting conditions.

two formulations are bioequivalent, and the tolerability of both products is acceptable.

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Conflict of Interest

The authors declare no conflict of interest.

Statement of Contribution of Researchers

The manuscript was written by F. Y. and S. P. A. The test formulation was developed by F. Y. and A. A. The clinical study protocol and report were written by Ö. A., S. K., A. P., and M. Ö. K. The bioequivalence study was conducted by O. S. and S. P. A.. The statistical analyses were performed by F. Y. and O. S.

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