Comparative Bioequivalence Study of Two Marketed Formulation of Betamethasone Injectable Suspensions

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Key words
- bioequivalence
- betamethasone injectable suspension
- pharmacokinetics
- HPLC

Abstract

Background: The aim of this study was to determine the bioequivalence of generic (test) and branded (reference) formulations of betamethasone injectable suspension in healthy Iranian subjects for the purpose of meeting regulatory requirements for bioequivalence of the generic formulation in Iran.

Methods: 24 healthy Iranian male volunteers were participated for this single-dose, randomized, open label, 2 period crossover study separated by a 2-week washout period. For the assessment of betamethasone, blood samples were obtained before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration. Plasma concentration of betamethasone was analyzed with a simple, rapid and validated high performance liquid chromatography method with ultraviolet detection. Pharmacokinetic parameters, representing the rate (C_{max}, T_{max}), and the extent (AUC_{0-t} and $AUC_{0-\infty}$) of betamethasone absorption were calculated and analyzed for 2 formulations. The 2 formulations were to be considered bioequivalent if the 90% confidence intervals (CI)s for the logarithm-transformed values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ fell within the predetermined range of 80–125%.

Results: The 90% CIs of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 85.4–104.4%, 96.2–112.1% and 98.3–115.8%, respectively.

Conclusion: Based on the 90% CIs of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ in these healthy Iranian male subjects, the test and reference formulations of betamethasone injectable suspension met the regulatory requirements for bioequivalence.

Introduction

Glucocorticoids, natural and synthetic, are adrenocortical steroids readily absorbed from the gastrointestinal tract. Naturally occurring glucocorticoids (hydrocortisone and cortisone), are used as replacement therapy in adrenocortical deficiency states. Their synthetic analogues are primarily used for their anti-inflammatory effects in disorders of many organ systems. A derivative of prednisolone, betamethasone (BMZ), has a 16β-methyl group that enhances the anti-inflammatory action of the molecule. BMZ sodium phosphate (CAS No: 151-73-5), a soluble ester, provides rapid activity, while BMZ acetate (CAS No: 987-24-6) is only slightly soluble and supplies sustained activity [1–6]. Betamethasone injectable suspension is a sterile aqueous suspension containing BMZ 3 mg/mL as BMZ sodium phosphate, and BMZ acetate 3 mg/mL. When oral therapy is not possible, the intramuscular use of BMZ sodium phosphate and BMZ acetate injectable suspension is indicated in allergic states, dermatologic diseases, endocrine disorders, rheumatic disorders, etc [5,7–10]. Many countries have established procedures for the introduction of generic pharmaceutical formulations. In order to protect patients, these generic formulations must be demonstrated to be therapeutically equivalent to an innovator formulation. Bioequivalence means the absence of a significant difference in the rate and extent of the active ingredient. The fundamental reason for performing bioequivalence testing is to ensure, as far as possible, that there are not any important differences in safety and efficacy between a generic and an innovator drug formulation and the formulations are therapeutically equivalent [11–13]. The present study was designed to investigate the bioequivalence of generic (Exir) and branded formulations of BMZ in healthy subjects for the purpose of meeting regulatory requirements for marketing the generic formulation in Iran.

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Materials and Methods

Materials

Methanol and dichloromethane were HPLC grade and purchased from Merck Company (Darmstadt, Germany). Hydrocortisone and betamethasone powders were provided form Fluka Chemica (Milano, Italy). The test formulation was betamethasone injectable suspension manufactured by Exir, Boroujerd, Iran with a batch number of 0150605, and the reference formulation was from respected manufacturer with a batch number of 5AHUB02A02 respectively.

Methods

Study design

24 healthy male volunteers who had given written informed consent were enrolled in the trial and completed both treatment periods. The mean ± SD age was 24.4 ± 3.1 years (20–33 years) and the mean ± SD body weight was 71.0 ± 7.3 Kg (60–89 Kg). To qualify the study, subjects had to be in acceptable physical condition as evidenced by absence of abnormal findings on physical examination. The mercury sphygmomanometer device was used for blood pressure measurement. When taking the measurement from the right upper arm, the arm was supported so that the cuff was at the level of the right atrium with the arm straight and the antecubital fossa faces upward. The radial artery was used as the pulse point to determine subjects' heart rate. The study used an open label, randomized, 2 period and cross over design. The 2 treatment days were separated by a 2-week washout period. This study was carried out in accordance with the principles of the World Medical Association's Declaration of Helsinki and its amendments and was approved by the local ethical committee of Tabriz University of Medical Sciences (5/4/2402).

The volunteers received an intramuscular injection of either long acting formulation containing betamethasone (as disodium phosphate) equivalent to 3 mg/1 mL and betamethasone acetate 3 mg/1 mL. Inactive ingredients per mL of each formulation include 7.1 mg dibasic sodium phosphate; 3.4 mg monobasic sodium phosphate; 0.1 mg edetate disodium; and 0.2 mg benzalkonium chloride as preservative. The pH was between 6.8 and 7.2. Blood samples (7 mL) for pharmacokinetic evaluations were obtained just before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after dosing during each treatment period. Samples were centrifuged at 5 000 for 5 min. Plasma samples were separated and stored at −20 °C until analysis. The time interval between collecting the blood samples and their analysis was less than 1 month. Results of preliminary study confirmed the stability of drug during this time in frozen samples. For drug analysis frozen samples were thawed at room temperature and then 50 µL of Internal Standard (IS) solution (Hydrocortisone acetate, 500 ng/mL) and 5 mL of dichloromethane were added to 1 mL of plasma. The solution was centrifuged at 5 000 rpm for 5 min and then dichloromethane phase was separated and evaporated at 45 °C. Residue was dissolved in 250 µl of mobile phase and 200 µL of it was injected into chromatographic column. A high performance liquid chromatographic method previously developed by Petersen et al. [14], was used for plasma drug concentration measurement. Liquid chromatographic system (Shimadzu, Japan) comprising a variable wavelength ultraviolet spectrophotometric detector set at 254 nm was used. Separation column was Shimpack CLC C8 (5 µm, 250 × 4.5 mm) (Shimadzu, Columbia, MD). Mobile phase consisted of methanol and water, 65:35 (v/v %). At flow rate of 1.5 mL/min, retention time for BMZ and IS was 4.9 and 5.5 min respectively. A calibration curve was established from analyzing spiked samples, which are obtained by the addition of known quantities of BMZ to plasma. The standard concentrations of BMZ were 1.56, 3.125, 6.25, 12.5, 18.75 and 25 ng/mL. The peak area ratio of BMZ to IS was plotted against the standard sample concentrations. The precision and accuracy of the method were assessed in plasma by performing replicate analysis of quality control (QC) samples at 4 levels (5, 10, 15, and 25 ng/mL), 4 time in a day and in 4 consecutive days. Extraction recovery of BMZ from plasma samples were determined for 3 QC concentrations (6.25, 12.5 and 25 ng/mL) by comparing the peak area obtained from the plasma with the peak area obtained from the direct injection of pure prepared standard solution. Sensitivity parameters, LOD and LOQ were calculated as signal to noise ratios of 3 and 10 respectively [15–19].

A non-compartment pharmacokinetic model was used to obtain pharmacokinetic parameters. The analyzed parameters were the area under the plasma concentration-time curve (AUC), peak plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}). The individual subjects' C_{max} was obtained directly from the concentration-time curve, and AUC_{0-t} was determined using the trapezoidal rule. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/k_e$, where C_t was the last measurable plasma drug concentration and k_e was the elimination rate constant, obtained as the slope of the linear regression of the log-transformed concentration-time curve data in the terminal phase. The $T_{1/2}$ was calculated as $\ln 2/K_e$. Parametric 90% confidence intervals (CIs) based on the ANOVA of the mean test/reference ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were determined. If the differences in parameters between the 2 formulations were not statistically significant and the 90% CIs for each parameter was within the predetermined range of 80–125%, the 2 drugs were to be considered bioequivalent based on US Food and Drug Administration (FDA) guidelines [11–13, 17, 20].

Results and Discussion

Several analytical methods such as gas chromatography-MS, liquid chromatography (LC) – tandem mass spectrometry (MS), micellar electrokinetic capillary chromatography (MEKC), Differential-pulse-polarography (DPP), Radioimmunoassay and HPLC method have been used previously to determine BMZ in numerous biological and pharmaceutical samples, e.g., urine, serum, plasma, milk, tissues and different drug formulations [15, 16, 21–27]. The HPLC method used in this study was previously reported by Petersen et al. This method demonstrated good sensitivity and specificity for BMZ measurements. The used method was linear in the concentration range of 1.56–25 ng/mL, with $r^2 = 0.995$. The lower limit of detection and quantitation for analyte were 0.3 and 1 ng/mL. Representative chromatogram of a typical blank plasma sample and different betamethasone standard concentrations in plasma samples are illustrated in **Fig. 1**.

Reproducibility of the measurement was evaluated by inter-day and intra-day analyses and illustrated by the accuracy (error) and the precision (coefficient of variation, CV), as shown in **Table 1**. The precisions were all < 10%. The accuracy was between 101.44% and 116.90%. The results of the reproducibility for both intra-day and inter-day were within an acceptable range. The recovery of the method was between 83.83% and 88.68% (**Table 2**).

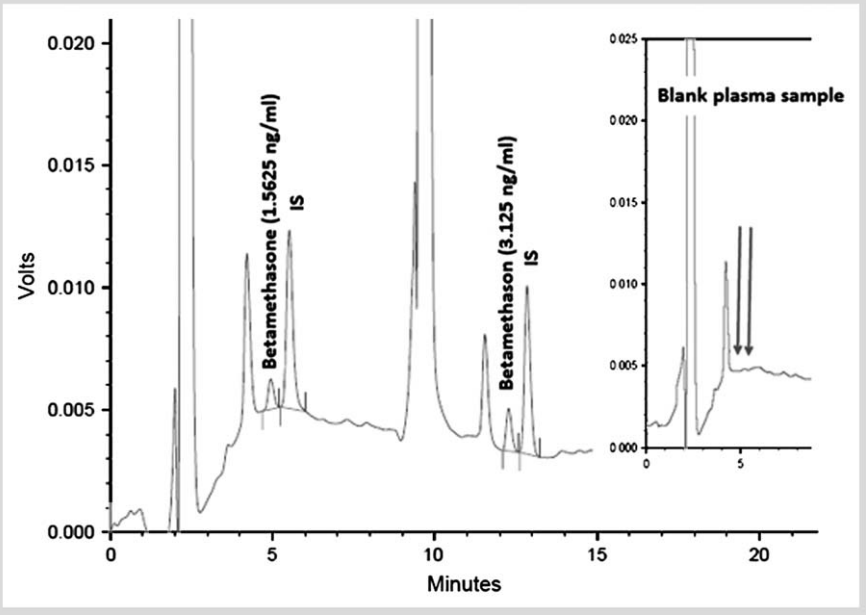

Fig. 1 Representative chromatogram of a typical blank plasma sample and different betamethasone standard concentrations in plasma samples.

Table 1 Intra-day and inter-day precision and accuracy obtained from 4 levels of QC samples.

Added concentration	Intra-day				Inter-day			
(ng/mL)	Mean (ng/mL)	SD (ng/mL)	CV (%)	Accuracy (%)	Mean (ng/mL)	SD (ng/mL)	CV (%)	Accuracy (%)
5	5.85	0.44	7.56	116.90	5.62	0.55	9.84	112.45
10	10.74	0.49	4.54	107.38	10.71	0.91	8.48	107.13
15	15.50	0.68	4.40	103.35	15.12	0.47	3.12	100.78
25	25.48	0.91	3.58	101.91	25.36	0.84	3.30	101.44

Table 2 Extraction recovery of betamethasone from plasma samples.

Concentration (ng/mL)	6.25	12.5	25
Mean Recovery (%)	83.83	88.68	84.89
SD	2.18	2.57	3.98
RSD	2.60	2.89	4.69

In this investigation the mean concentration-time profiles of the test and reference formulations were not significantly different (**Fig. 2**). In a previous study, Salem et al. reported that after single-dose intramuscular administration of a suspension formulation containing 3 mg BMZ acetate and 3 mg BMZ phosphate, C_{max} and $AUC_{0-\infty}$ values were 21.19 (±20.29) ng/mL and 354.06 (±76.28) ng.h/mL. In another study by Salem et al. in 2012, the reported values for AUC_{0-t}, $AUC_{0-\infty}$, T_{max} and C_{max} were 466.51 (±115.11) ng.h/mL, 506.95(±125.03) ng.h/mL, 1.56 (±1.32) h and 33.21(±8.71) ng/mL respectively [7,28].

Also in Petersen et al.'s study, $AUC_{0-\infty}$, T_{max} and C_{max} were 486.33 ng.h/mL, 1.55 h and 55 ng/mL [29]. However in our study the mean (±SD) C_{max} values, were 22.28 (±6.24) ng/mL and 23.81 (±4.72) ng/mL for the test and reference formulation respectively. Corresponding data for T_{max}, were 1.77 (±0.44) and 1.44 (±0.56) h. The AUC_{0-t} values were 140.21 (±46.58) ng.h/mL and 138.96 (±52.02) ng.h/mL for the test and reference formulation. Corresponding $AUC_{0-\infty}$ values were 145.75 (±48.26) ng.h/mL and 141.42 (±54.99) ng.h/mL respectively (**Table 3**).

ANOVA test for these parameters after log-transformation of the data found no statistically significant differences between the 2 formulations in period, product or group. Results of relative bioavailability (T/R) were 95% (90% CI, 85.4–104.4%) for C_{max}, 104% (90% CI, 96.2–112.1%) for AUC_{0-t} and 107% (90% CI, 98.3–115.8%) for $AUC_{0-\infty}$. These 90% CIs met the criterion for bioequivalence (**Table 4**, **5**).

Conclusion

The mean and standard deviation of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the test formulations did not differ significantly, suggesting that the blood profiles generated by test formulation were comparable to reference formulation. ANOVA for these parameters, after log-transformation of the data, showed no statistically significant difference between the 2 formulations either in periods, product or group, having a P-value greater than 0.05. The 90% CIs also demonstrated that the ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the 2 formulations lie within the FDA acceptable range of 80–125%.

Based on the obtained results we can conclude that betamethasone injectable suspension, manufactured by Exir Pharmaceutical Company, Boroujerd, Iran is bioequivalent to reference formulation, and both formulations can be considered equally effective and interchangeable in medical practice.

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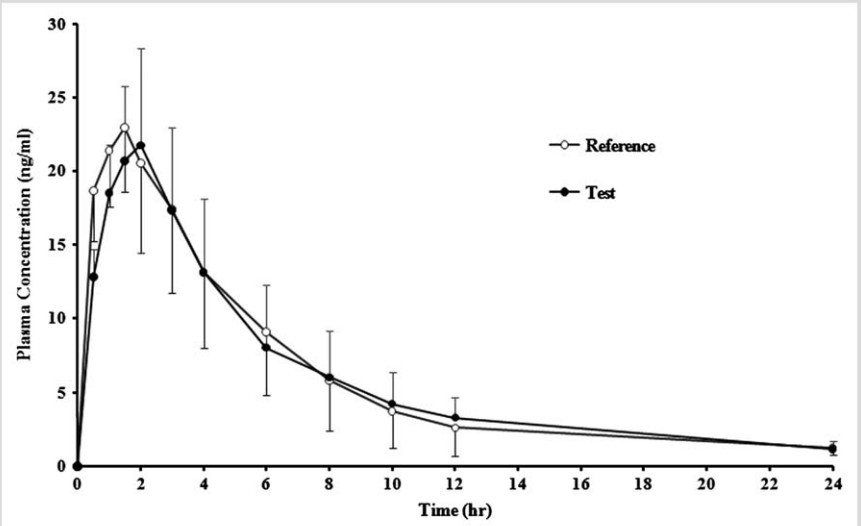

Fig. 2 Average (±SD) plasma concentration of betamethasone vs. time plots of 24 volunteers after administration of test and reference formulations.

Table 3 Pharmacokinetic parameters for the test and reference preparations after intramuscular injection to 24 healthy volunteers.

Parameter	C_{max} (ng/mL)		T_{max} (h)		$T_{1/2}$ (h)		AUC_{0-t} (ng.h/mL)		$AUC_{0-\infty}$ (ng.h/mL)	
	T	R	T	R	T	R	T	R	T	R
Mean	22.28	23.81	1.77	1.44	4.10	3.03	140.21	138.96	145.75	141.42
SD	6.24	4.72	0.44	0.56	1.96	0.99	46.58	52.02	48.26	54.99
RSD	28.00	19.81	24.95	38.80	47.76	32.52	33.22	37.44	33.11	38.88
Lower CI (90%)	20.19	22.23	1.62	1.25	3.45	2.70	124.57	121.49	129.54	122.96
Upper CI (90%)	24.38	25.40	1.92	1.62	4.76	3.36	155.86	156.43	161.95	159.88

Table 4 Ratios (T/R) of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and difference of the T_{max} values.

Parameter	C_{max} ratio (T/R)	AUC_{0-t} ratio (T/R)	$AUC_{0-\infty}$ ratio (T/R)	T_{max} difference (T-R)
Mean	0.95	1.04	1.07	0.33
SD	0.28	0.24	0.26	0.64
RSD (%)	29.85	22.75	24.32	191.11
Lower CI (90%)	0.85	0.96	0.98	0.12
Upper CI (90%)	1.04	1.12	1.16	0.55

Table 5 The 90% CI for the ratios T/R of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values.

Pharmacokinetic parameter	ANOVA (P-value) Variation source			CI 90% for the ratios
	Product	Group	Period	
C_{max}	0.1935	0.3428	0.2504	85.4–104.4
AUC_{0-t}	0.8498	0.4331	0.7467	96.2–112.1
$AUC_{0-\infty}$	0.5409	0.4200	0.5630	98.3–115.8

Conflict of Interest

The authors indicate that they have no conflicts of interest in this report.

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