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Pharmacokinetics and Bioavailability Study of a Prednisolone Tablet as a Single Oral Dose in Bangladeshi Healthy Volunteers

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Abstract

The aim of the study was to assess the pharmacokinetic and bioavailability of 2 formulations of 5-mg prednisolone tablets, reference product (Teva UK Limited) and Pred (Eskayef Bangladesh Ltd) as test product. The open-label, randomized, 2-way crossover studies were conducted on 14 healthy subjects. Participants were assigned to receive both products as a single dose (20 mg formulations, 4 × 5 mg tablets) followed by a 2 weeks' washout period. Following oral administration, samples were obtained at various time intervals and analyzed for prednisolone concentrations using a validated high-performance liquid chromatography assay method with ultraviolet detection. The obtained values for test and reference products were 683.00 ± 94.54 ng/mL and 635.16 ± 125.57 ng/mL for C_{max}; 2716.54 ± 196.28 ng·h/mL and 2780.5 ± 119.73 ng·h/mL for $AUC_{0\text{-}12}$; 3284.36 ± 138.12 ng·h/mL and 3317.96 ± 133.95 ng·h/mL for $AUC_{0\text{-}\infty}$, respectively. From the paired Student *t* test, no significant differences between 2 formulations were observed ($P > .05$). The 90% confidence intervals of C_{max}, $AUC_{0\text{-}12}$, and $AUC_{0\text{-}\infty}$ were found to be 99.0% to 100.9%, 99.4% to 100.5%, and 99.9% to 101.3%, respectively. Finally, it can be concluded that Pred (Test) of Eskayef Bangladesh Ltd and prednisolone (Reference) of Teva UK Limited are bioequivalent and interchangeable.

Keywords

prednisolone, pharmacokinetics, bioavailability, bioequivalence, Bangladeshi healthy volunteers.

Introduction

Prednisolone is a well-known corticosteroid used extensively for its anti-inflammatory and immunosuppressive properties.[1] It is a potent synthetic glucocorticoid that is the active metabolite of its prodrug prednisone.[2] Chemically, it is known as 11βb, 17, 21-trihydoxypregna-1,4-diene-3,20-dione, CAS 50-24-8.[1] Prednisolone has been listed by World Health Organization as an essential medicine.[3] It is used to treat a myriad of acute and chronic diseases, including asthma, hepatitis, arthritis, systemic lupus erythematosus, and allergic dermatitis. Compared to hydrocortisone, prednisolone exhibits significantly higher anti-inflammatory potency with lower sodium retaining ability.

The mechanism of action of prednisolone is via activation of a cytoplasmic glucocorticoid receptor and subsequent nuclear translocation. In the nucleus, the prednisolone–glucocorticoid receptor complex binds to specific DNA- binding sites known as glucocorticoid response elements (GREs) resulting in gene expression or inhibition. Complex binding to positive GREs leads to synthesis of anti-inflammatory proteins while binding to negative GREs block the transcription of inflammatory genes.[4]

In humans, the pharmacokinetics of prednisolone is complex. After oral intake, it exhibits rapid absorption and becomes almost completely (80%-100%) available. Peak plasma concentrations reach 1 to 2 hours after oral administration.[5,6] Distribution of prednisolone is dependent on its protein-binding properties. Prednisolone binds reversibly to albumin as well as to a specific α1-glycoprotein named transcortin (corticosteroid-

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binding globulin). Transcortin has a higher affinity but a relatively low capacity for binding prednisolone and thus becomes saturated at therapeutic concentrations[7] because of its relatively lower abundance in plasma. On the contrary, albumin possesses a lower affinity but a much higher capacity for binding prednisolone and does not appear to become saturated.[8] To summarize, prednisolone shows higher protein binding (80%-90%) at low concentrations, but lower protein binding (60%-70%) at higher concentrations as transcortin becomes saturated.[9]

Similar to many steroid hormones, prednisolone is thought to be metabolized by cytochrome P450 enzyme, specifically CYP3A4.[10] The main metabolic pathways are 6-hydroxylation by CYP3A4 along with reduction in 20-keto group of both prednisolone and prednisone.[11] Excretion of prednisolone occurs by urination, and roughly 20% of dose gets excreted in its unchanged form.[11] Extensive use of generic drug products to minimize the health-care costs has been the dominant trend observed during the last 4 decades. However, clinical use of generic formulations depends on their similarity in physicochemical properties as well as their bioequivalence with reference drug products. Publication of bioequivalence data of commercially available generic drugs is important in order to provide doctors with reassurance that a specific generic drug provides the expected therapeutic effect. In Bangladesh, there are 22 generic formulations of prednisolone, so interchangeability with internationally recognized brands has been a great concern. We, therefore, aimed to conduct a human in vivo pharmacokinetics and bioequivalence study testing a prednisolone 5-mg tablet (Pred, produced by Eskayef Bangladesh Ltd, Dhaka, Bangladesh) against a chosen reference drug (5 mg prednisolone tablet, produced by Teva UK Limited, Ridings Point, Whistler Drive, Castleford, United Kingdom) in Bangladeshi healthy volunteers for the very first time.

Materials and Methods

Participant Selection

A total of 14 healthy participants (7 males and 7 females) were enrolled into the study with mean age (standard deviation [SD]), 23.12 (2.07) years (range: 21-28 years); mean body weight (SD), 56.93 (8.16) kg (range: 48-72 kg); mean height (SD), 1.64 (0.07) m (range: 1.52-1.72 m); and mean body mass index (SD), 21.05 (2.36) kg/m^2 (range: 20.52-27.21 kg/m^2). A minimum number of 12 evaluable participants should be included in any bioequivalence study, although 18 to 24 participants are used to increase the statistical power.[12] A power analysis ($\beta = .2$) determined that the power of the analysis of variance (ANOVA) was ≥ 0.8 at a 90% confidence interval (CI), indicating that the number of participants ($n = 14$) enrolled in the study was sufficient.

Participants were assessed by a clinical screening procedure prior to selection for the study. A recent medical history was taken, and in-depth physical examination was performed. Moreover, vital sign measurements, chest X-ray, electrocardiography (ECG), and routine blood and urine analysis data were obtained for all participants within 7 days prior to commencement of the study. Participants with no evidence of clinically significant abnormal hematological, serum chemistry, and urine analysis values were finally selected for the study. All the participants were informed of the experimental procedure with aims and risks and signed their written consent.

Exclusion criteria included hypersensitivity to any steroid drug, requirement of chronic medication (eg, theophylline, antacid, glibenclamide, phenytoin, iron, or vitamins), abnormal gastrointestinal condition which may affect the absorption and distribution of prednisolone, donation of blood, or use of an investigational agent within 30 days preceding the first dose of the study.

History and/or clinical evidence of significant cardiovascular, respiratory, hematological (including pancytopenia), neurological, dermatologic, gastrointestinal (including bleeding or ulcers), or hepatic disease were also considered as exclusion criteria.

All participants were requested to abstain from taking any medication (including over-the-counter drugs) for at least 1 week prior to the study and until after it is completed. They were also asked to refrain from smoking and taking alcohol or caffeinated products for a minimum of 48 hours prior to and throughout the study.

Study Design

The study was a single-dose, randomized, open-label, 2×2 crossover design with a 2 weeks' washout period. It was carried out in the Faculty of Pharmacy, Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Bangladesh in collaboration with Dhaka University Medical Center. Approval of study protocol was given by the ethics committee of Bangladesh Medical Research Council (BMRC) with an ethics code of BMRC/ERC/2008/14-599. The principles of the International Conference of Harmonization guideline for Good Clinical Practice, in accordance with Declaration of Helsinki and its further amendments, were implemented throughout the study procedure.[13] Each participant received a single oral dose (20 mg, 4×5 mg tablets) of either formulation (test or reference product) administered with 250 mL of water after a period of overnight fast. They were served standardized breakfast and lunch at 4 and 8 hours after drug intake. Throughout the experiment, 2 registered physicians monitored the condition of the participants to take care of any possible adverse events at all times.

Tolerability

Tolerability was checked by careful monitoring of vital signs (blood pressure, heart rate, body temperature, and so on) at baseline, 4 hourly intervals during the study and at the end of each period by expert clinicians. A complete physical examination was also carried out before and 24 hours after drug administration. Participants were interviewed for any sort of

physical or mental discomfort throughout the study period and also after 2 weeks of drug administration one of either formulation at the second period. All the participants were advised to report any occurrence of adverse events at any time during the experiment.

Blood Sampling

Median cubital vein was cannulated using a 20-G ×1.25-inch catheter (Vasofix Braunüle, B. Braun Melsungen AG, Melsungen, Germany), and heparinized normal saline injectable solution was flushed into the cannula to prevent blood clotting. Approximately 3 mL of venous blood samples were collected via the cannula at the following times points: prior to dosing 0 (baseline), 0.5, 1.0, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 12.0 hours after dosing. Before each blood collection, to remove preinjected heparinized saline, 2 mL of blood was discarded from the inserted catheter. The blood samples were left for 30 minutes at room temperature in a dark place and then centrifuged at 865*g* for 15 minutes at 25°C. Collected serum was stored at −80°C until further analysis.

Sample Preparation

Prednisolone was extracted from human serum samples by protein precipitation method. Briefly, 500 µL aliquot of serum sample was taken in a polypropylene centrifuge tube and into that sample 1.5 mL deionized water, 100 µL 0.5% phosphoric acid to extract membrane integral protein and to maintain the solubility of hydrophobic proteins, and 3 mL ethyl acetate to remove detergent were added. The tube was tightly capped and vigorously shaken (vortex mixer) for 5 minutes before being centrifuged at 1538*g* for 10 minutes. The organic phase was then aspirated and transferred to new 12-mL centrifuge tubes and washed with 250 µL 0.1 mol/L sodium hydroxide, followed by a 5-minute sonication and a 10-minute centrifugation at 1538*g*. The organic phase was transferred to 5-mL glass tubes and evaporated to dryness under a gentle nitrogen stream. The remaining dried residue was reconstituted with 700 µL of methanol and sonicated for 10 minutes. Then, 20 µL of the reconstitute was injected into the chromatographic system.

Chromatographic Analysis

A Shimadzu (Columbia, Maryland) high-performance liquid chromatography system was used in quantification of prednisolone, which consists of an SCL-10Avp system controller and 2 LC-8A pumps. Data acquisition and processing were performed using LC solution (Version 1.03 SP3; Shimadzu Corporation, Columbia, MD) software running under Windows XP on a Pentium PC. Ultraviolet detection was achieved with a SPD-10Avp UV-VIS detector (Shimadzu Corporation) at 254 nm. To obtain the calibration curve, a series of prednisolone solutions in serum with concentrations from 25 to 1500 ng/mL were prepared (n = 8). The mobile phase consisted of 0.2 mol/L potassium dihydrogen phosphate buffer (pH 3.55 ± 0.1 adjusted with 10 mol/L solution of acetic acid) and acetonitrile (70:30) passed through XTerra C8 column (5 µm, 4.6 × 250 mm; Waters, Massachusetts) at room temperature at a flow rate of 1.0 mL/min.

Pharmacokinetic Analysis

A noncompartmental approach was followed to measure pharmacokinetic properties, from serum concentrations of prednisolone, using software Kinetica (version 4.4.1; Thermo Electron Corporation, United Kingdom). C_{max} was estimated directly from the observed concentrations and T_{max} as the corresponding time point for the occurrence of C_{max}. Linear trapezoidal method was used to calculate $AUC_{0\text{-}t}$ until the last measurable serum prednisolone concentration, and $AUC_{0\text{-}\infty}$ was calculated using the formula, $AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_{last}/K_{el}$. K_{el} was the terminal elimination rate constant. It was calculated by linear least square regression of the last 3 to 4 time points in the log concentration–time profile. The terminal half-life was calculated by the following equation: $t_{1/2} = 0.693/K_{el}$. The mean residence time (MRT) was calculated as:

$$MRT = AUMC_{0-\infty}/AUC_{0-\infty}.$$

Statistical Analysis

Paired *t* test was used to evaluate the difference between all parameters at 5% level of significance. Moreover, ANOVA was performed to evaluate the source of variations. Following logarithmic transformation of pharmacokinetic parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$), large sample-based 90% CIs were analyzed for the assessment of bioequivalence in accordance with the current Food and Drug Administration (FDA) guidelines.[13]

Results

Method Validation

The analytical method used for quantification provided the appropriate specificity, sensitivity, accuracy, and precision. The calibration curve was found to be linear over the concentration range of 25 to 1500 ng/mL with regression coefficient of 0.999. The limit of quantification was found to be the lowest concentration on the calibration curve (25 ng/mL) for which an acceptable accuracy of 108.7% and a precision of 2.75% were obtained. The intraday and interday precision of the method used to measure serum prednisolone concentration ranged from 3.79% to 7.55% and 5.72% to 9.60%, respectively. The average recovery of prednisolone from serum was 97.5%.

Tolerability

Both the formulations were well tolerated. All 14 participants completed the study without reporting any adverse effects. There was no evidence of clinically significant abnormalities

Table 1. Mean (SD) Serum Pharmacokinetic Parameters of All the Volunteers for the Test and Reference Formulations.

Pharmacokinetic Parameters	Test (n = 14)	Reference (n = 14)	*P* Value
C_{max}, ng/mL	683.00 (94.54)	635.16 (125.57)	.2636
T_{max}, h	2.27 (0.07)	2.21 (0.09)	.0597
$AUC_{0\text{-}12}$, ng·h/mL	2716.54 (196.28)	2780.50 (119.73)	.3075
$AUC_{0\text{-}\infty}$, ng·h/mL	3284.36 (138.12)	3317.96 (133.95)	.4977
K_{el}, h^{-1}	3.62 (0.39)	3.32 (0.43)	.0641
$AUMC_{0\text{-}12}$, ng·h^2/mL	10 164.79 (466.03)	10 368.89 (512.72)	.2805
$AUMC_{0\text{-}\infty}$, ng·h^2/mL	16 868.61 (859.80)	16 786.76 (831.91)	.8000
$t_{1/2}$, h	3.30 (0.23)	3.16 (0.14)	.0626
MRT, h	5.49 (0.21)	5.36 (0.14)	.0649

Abbreviations: MRT, mean residence time; SD, standard deviation.

on physical examination. Vital signs measurements, ECG recordings, and laboratory results were also found to be normal for all the participants.

Pharmacokinetic Properties

A summary of mean (SD) serum prednisolone pharmacokinetic parameters is listed in Table 1. Calculated C_{max} (mean [SD]) for test and reference formulations are 683.00 [94.54] and 635.16 [125.57] ng/mL, respectively. They were reached at mean T_{max} of 2.27 and 2.21 hours, respectively. The $AUC_{0\text{-}t}$–$AUC_{0\text{-}\infty}$ ratio for all the participants was >80%. Test and reference formulations had a mean elimination half-life of 3.30 and 3.16 hours, respectively. Figure 1 depicts the mean (SD) serum prednisolone concentration-time curves for both formulations.

Results of Statistical Analysis

The statistical analysis results for all the pharmacokinetic parameters are presented in Table 1. All the *P* values were found to be >.05 which indicates that there was no statistically significant difference between the test and the reference formulation.

Aanalysis of variance was used to measure the effect of formulations, sequences, periods, sex, and subjects on pharmacokinetic parameters.[6] Sequence effect was assessed against the between-patient mean squares. Within-patient mean error was used to test all other factors. There was no significant formulation, period, sequence, or gender effect on any of the pharmacokinetic parameters ($P > .05$; Table 2). On the contrary, significant differences were observed for $AUC_{0\text{-}12}$, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-}\infty}$, $t_{1/2}$, and k_{el} only in case of patient variation. This may be attributed to the interindividual variations between participants. All the parameters tested in this experiment represent the rate and extent of absorption of the drug from its dosage form and so the statistically not significant differences between 2 formulations.

The 90% CI for prednisolone C_{max}, $AUC_{0\text{-}12}$, and $AUC_{0\text{-}\infty}$ were 99.0% to 100.9%, 99.4% to 100.5%, and 99.9% to 101.3%, respectively. Each pharmacokinetic parameter value was found to be within the predetermined range of 80% to 125% that fulfills the FDA requirement for bioequivalence

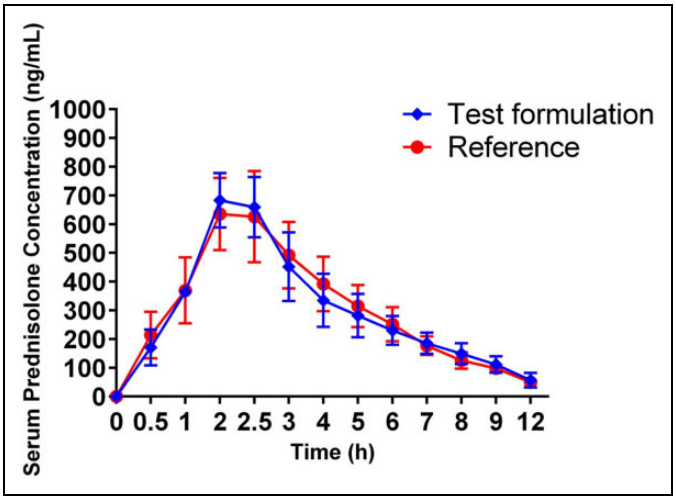

Figure 1. Mean (standard deviation [SD]) prednisolone concentration-versus-time curves over 12 hours in adult healthy Bangladeshi volunteers (n = 14).

(Table 3).[13] So these 2 drugs are therapeutically equivalent to each other.

Discussion

The purpose of this study was to compare the bioavailability and tolerability of 2 formulations of prednisolone tablets in Bangladeshi healthy participants. According to the guidelines of US FDA, 2 drug formulations are considered bioequivalent when they are pharmaceutically equivalent as well as their bioavailability profiles are so similar that the possibility to produce clinically relevant differences (tolerability and efficacy) is very low.[14] Our results showed no statistically significant differences between the test and the reference products as indicated by C_{max} and AUC comparisons as well as the plasma concentration–time curves. Ninety percent CIs for all vital pharmacokinetics parameters were found to be within the FDA-recommended range to qualify for bioequivalence. Both formulations were well tolerated, and all 14 volunteers completed the experiment without any occurrence of adverse effects. This indicates that the 2 formulations are bioequivalent and hence may be regarded as interchangeable.

Table 2. *P* Values for Sources of Variations Obtained From Analysis of Variance (ANOVA).

Sources of Variations	C_{max}, ng/mL	T_{max}, h	AUC_{0-12}, ng·h/mL	$AUC_{0-\infty}$, ng·h/mL	K_{el}, h^{-1}	$AUMC_{0-12}$, ng·h^2/mL	$AUMC_{0-\infty}$, ng·h^2/mL	$t_{1/2}$, h	MRT, h
Formulation	0.969	0.523	0.674	0.479	0.101	0.068	0.947	0.139	0.791
Period	0.276	0.401	0.393	0.371	0.172	0.346	0.447	0.736	0.223
Sequence	0.237	0.684	0.726	0.852	0.127	0.294	0.128	0.593	0.762
Participants	0.141	0.084	0.012	0.013	0.039	0.956	0.014	0.026	0.062
Sex	0.812	0.523	0.089	0.166	0.128	0.631	0.226	0.525	0.335

Abbreviation: MRT, mean residence time;

Table 3. 90% Confidence Intervals for Different Pharmacokinetic Parameters.

	90% Confidence Intervals		
Parameters	Point Estimate	Upper Limit	Lower Limit
C_{max}	99.9	99	100.9
AUC_{0-12}	100.3	99.4	100.5
$AUC_{0-\infty}$	99.5	99.9	101.3

Prednisolone is rapidly available when taken orally. Luippold et al, who investigated the oral bioavailabilities in 13 participants using different formulations of 20 mg prednisolone tablets, reported that the time to reach maximum plasma prednisolone concentration (T_{max}) was approximately 1 hour.[9] Leclercq and Copinschi, on the other hand, found a significant prolongation of T_{max} for the same oral dose of prednisolone which is in accordance with present study.[15] Similar conclusions were also contemplated by Sandberg et al and Tanner et al.[5,16] Moreover, values of C_{max} were found similar as compared to previous study.[17] Biological half-life of prednisolone is in the range of 2.1 to 3.5 hours.[18] In the present study, we found the mean elimination half-life of prednisolone 3.30 hours for test formulation and 3.16 hours for reference formulation which are slightly longer than observed in a previous study[17] but are comparable with the data reported by other studies.[5,19,20]

Sex acts as an influential factor in the pharmacokinetics and pharmacodynamics of drugs.[21] Our study included 7 female participants and didn't find any significant difference in any of the investigated pharmacokinetic parameters. Values of MRT based on sex also revealed no significant variation: female group ($n = 7$, Test (SE) $= 4.98 \pm 0.53$, Ref (SE) $= 4.62 \pm 0.49$; *P* value >.05); male group ($n = 7$, Test (SE) $= 5.12 \pm 0.61$, Ref (SE) $= 4.77 \pm 0.56$; *P* value >.05) Standard error (SE).

The current study had some limitations. Further studies are required to find the effects of age variation, drug–drug interactions, and food intake on bioavailability of prednisolone. To avoid minor problems related to interindividual variation in pharmacokinetics of prednisolone, this study included healthy participants only.

Conclusion

To the best of our knowledge, this is the first bioequivalence study of a generic prednisolone formulation conducted in healthy Bangladeshi volunteers. Data from this study will help clinicians to become more assured about prescribing this generic drug without any interchangeability issue. Hopefully, this will reduce the health-care cost of the patients by allowing them to buy inexpensive generic prednisolone formulation instead of an expensive brand imported from foreign countries.

Authors' Note

T.B. and M.N.H.A. contributed equally to this work.

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Declaration of Conflicting Interests

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Supplemental Material

Supplementary material for this article is available online.

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