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Bioequivalence Evaluation of Two Brands of Aceclofenac 100 mg Tablets (Aceclofar and Bristaflam) in Healthy Human Volunteers

Naji Najib[a], Nasir Idkaidek[a], M. Beshtawi[a], Mohammed Bader[a], Isra' Admour[a], S. Mahmood Alam[b], Q. Zaman[b] and Ruwayda Dham[b,*]

[a] *International Pharmaceutical Research Centre (IPRC), Amman-Jordan*

[b] *Gulf Pharmaceutical Industries-Julphar, Dubai, UAE*

ABSTRACT: A randomized, two-way, crossover, bioequivalence study in 24 fasting, healthy, male volunteers was conducted to compare two brands of aceclofenac 100 mg tablets, Aceclofar (Julphar, UAE) as test and Bristaflam (Bristol Myers Squibb, Egypt) as the reference product. The drug was administered with 240 ml of water after a 10 h overnight fast on two treatment days separated by 1 week washout period. After dosing, serial blood samples were collected for a period of 24 h. Plasma harvested from blood was analysed for aceclofenac by a validated HPLC method with UV-visible detection capable of detecting aceclofenac in the range 0.2–8.0 μg/ml with the limit of quantitation as 0.2 μg/ml. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ_Z were determined from plasma concentrations for both formulations and found to be in good agreement with reported values. AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence interval (100.0%–106.4% for AUC_{0-t}, 100.2%–106.8% for $AUC_{0-\infty}$; 83.3%–102.8% for C_{max}) of test/reference ratio for these parameters were found to be within the bioequivalence acceptance range of 80%–125%. Based on these statistical inferences, it was concluded that Aceclofar is bioequivalent to Bristaflam. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: aceclofenac; bioequivalence; pharmacokinetics; HPLC; Julphar

Introduction

The bioequivalence of two formulations of the same drug denotes equivalence with respect to the rate and extent of their absorption. The area under concentration time curve (*AUC*) generally serves as the indicator for the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}), reflect the rate of absorption, especially in fast releasing drug formulations [1,2]. The present study was conducted to evaluate the bioequivalence of two brands of aceclofenac 100 mg tablets in fasting, healthy human volunteers.

Aceclofenac is a nonsteroidal antiinflammatory drug (NSAID) of the phenylacetic acid type that is structurally related to diclofenac [3]. Chemically it is $C_{16}H_{13}Cl_2NO_4$ with a molecular weight of 354.19 [3]. Aceclofenac has the following chemical structure.

Like other nonsteroidal antiinflammatory drugs, aceclofenac is a prostaglandin synthetase (cyclooxygenase) inhibitor, which decreases prostaglandin and leukotriene production, thereby inhibiting the inflammatory process. Aceclofenac has been shown to have potent antiinflammatory,

*Correspondence to: Gulf Pharmaceutical Industries, Julphar, Twin Towers 1201, P.O. Box 42040, Dubai, UAE.
E-mail: julphard@emirates.net.ae

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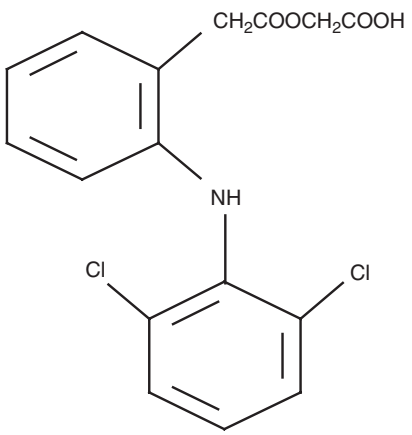

The chemical structure of aceclofenac [4]

analgesic, and antipyretic properties [5–9]. The usual dose of aceclofenac is 100 mg twice a day [8,10]. Other investigators have used either 75 mg 3 times a day [11] or 100 mg 3 times a day [12] in the treatment of rheumatoid arthritis and acute and chronic knee pain.

Aceclofenac is indicated for the acute and chronic treatment of the signs and symptoms of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and scapulohumeral periarthritis [5,13]. It is also indicated for pain of various aetiologies such as musculoskeletal pain (e.g. low back pain), dental pain or postsurgical pain.

Aceclofenac is absorbed rapidly as unchanged drug when taken orally, and its analgesic effect can begin within 30 min of ingestion [13]. It reaches a peak plasma concentration 1–3 h after ingestion [8]. C_{max} and *AUC* increase proportionally in the dose range 50–150 mg [13]. When aceclofenac is administered to fasting and fed healthy volunteers, only the rate but not the extent of aceclofenac absorption is affected by the presence of food in the gastrointestinal tract [13]. After absorption of aceclofenac, the drug is progressively hydrolysed to diclofenac in the circulation, which accounts for $<1\%$ of the activity. Aceclofenac is metabolized into a large number of compounds; the most important metabolite is 4-hydroxyaceclofenac [14]. The mean plasma elimination half-life is approximately 4 h; parent compound and its metabolites are eliminated primarily (66%) in the urine and, to a lesser extent, in the faeces [8].

Objectives of the study

The purpose of this study was to determine the bioequivalence of a new tablet formulation of aceclofenac (Gulf Pharmaceutical Industries, UAE) in comparison with Bristaflam (Bristol Myers Squibb, Egypt).

Materials and Methods

Study products

The test formulation was Aceclofar 100 mg tablet (Batch No. 0002, Expiry 09/2002) from Gulf Pharmaceutical Industries-Julphar, UAE and the reference product was Bristaflam 100 mg tablets (Batch No. D00794, Expiry 04/2003) manufactured by Bristol Myers Squibb, Egypt.

Study subjects

Twenty four healthy adult male volunteers participated in this study at Al-Mowasah Hospital, Amman, Jordan. The mean age was 21.75 ± 2.97 years, range 19–31 years, mean body weight was 72.6 ± 39.56 kg, range 54–87 kg, and the mean height was 174.46 ± 6.21 cm range 164–190 cm. The volunteers did not have any significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal or haematological diseases, as determined by their medical history, physical examination and routine laboratory tests (haematology, blood biochemistry and urine analysis). All subjects were negative for hepatitis B antigen and were instructed to abstain from taking any drug, including over-the-counter (OTC), for 2 weeks prior to and during the study period. They were informed about the aim and risks of the study by the clinical investigator, based on which they signed a written informed consent statement before entering the study. The study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practices. Before the start of the study the protocol was approved by the Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman, Jordan.

Drug administration and blood samples collection

The study was designed as a single dose, randomized, two treatment, two period cross-over. In the morning of phase I, after an overnight fast (10 h) volunteers were given a single dose of either formulation (reference or test) of aceclofenac 100 mg with 240 ml of water. No food was allowed until 5 h after dose administration; water intake was allowed after 2 h. Water, lunch and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored throughout the confinement period of study. They were not permitted to lie down or sleep for the first 5 h after the dose. Approximately 10 ml of blood samples for aceclofenac assay was drawn into heparinized tubes through an in-dwelling cannula before (0 h) and at 0.33, 0.66, 1.0, 1.33, 1.66, 2.0, 2.33, 2.66, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10, 12, and 24 h after dosing. Blood samples were centrifuged at 3500 rpm for 10 min, plasma was separated and kept frozen at –20°C until assayed. After a washout period of 7 days the study was repeated in the same manner to complete the crossover design.

Sample preparation for HPLC injection

A 100 µl internal standard (flufenamic acid 20 µg/ml) was added to 0.5 ml of plasma sample. The sample was vortexed for 30 s, 100 µl of 0.25 M phosphoric acid solution was added and vortexed for 30 s, then 7 ml of extraction solvent (diethyl ether) was added and vortexed for 2 min then centrifuged for 5 min at 3000 rpm. The supernatant (organic) layer was transferred to another 10 ml glass tube and evaporated to dryness in a water bath at 40°C, then reconstituted with 200 µl of mobile phase, vortexed for 1 min and transferred to an Eppendorf centrifuge tube (0.75 ml), centrifuged at 13 000 rpm for 2 min. A 100 µl of aliquot of sample was then injected on to the column and the peak area was recorded.

Chromatographic conditions

Plasma samples were analysed for aceclofenac according to a reported HPLC method [15], modified and validated before the study. All solvents used were of HPLC grade and were purchased from ACROS, USA while other chemicals and reagents were of analytical grade. Aceclofenac was obtained from Julphar, UAE; flufenamic acid was purchased from Sigma, Germany.

The HPLC system was from Shimadzu, Japan, and it consisted of a solvent delivery pump (LC-10ADvp), a system controller (SCL-10Avp), a manual injector Rheodyne injector (USA), and an UV-visible detector (SPD-10Avp). Integration was done using Class VP-5 software version 5.03. Chromatographic separation was performed using a Nova-Pak C_{18} (4 µm) (150 × 3.9 mm) HPLC cartridge column (Waters, USA). The mobile phase consisted of 43% acetonitrile and 57% 0.015 M sodium acetate trihydrate (pH of mobile phase was 4.60, adjusted with glacial acetic acid), and eluted at a flow rate of 1.5 ml/min at ambient temperature. The effluent was monitored using UV detection at 280 nm. The peak areas were measured, and the peak area ratios of drug to internal standard and the concentration were calculated by Class VP-5 software (version 5.03) Shimadzu. Each analysis required less than 12 min. The method was validated by following international guidelines [16].

Under the described conditions, the lower limit of quantitation was 0.2 µg/ml using 0.5 ml of plasma. The relationship between concentration and peak area ratio was found to be linear within the range 0.20–8.00 µg/ml. The intra-day accuracy of the method for aceclofenac ranged from 92.80% to 116.5%, while the intra-day precision ranged from 3.15% to 4.43%. The inter-day accuracy ranged from 95.40% to 109.7%, while the inter-day precision ranged from 4.04% to 12.32%. The absolute recovery was 65.52% while the relative recovery ranged from 98.86% to 112.50%. A stability study showed that aceclofenac was stable in plasma for 4 months when stored at −20°C.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of a model independent method using Kinetica™ 2000 computer program [17]. The elimination rate constant (λ_Z) was obtained as the

slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. Elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. Area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. Area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/\lambda_Z$, where C_t is the last measurable concentration.

Statistical analysis

For the purpose of bioequivalence analysis AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary variables. Two-way analysis of variance (ANOVA GLM procedure; KineticaTM 2000 Computer program [17]) for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. The difference between two related parameters was considered statistically significant for a p-value equal to or less than 0.05. Parametric 90% confidence intervals [18] based on the ANOVA of the mean test/reference (T/R) ratios of *AUCs* and C_{max} were computed.

Results and Discussion

Aceclofenac was well tolerated by all the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There were no drop-outs and all volunteers who started the study continued to the end and were discharged in good health.

Both formulations were readily absorbed from the gastrointestinal tract and aceclofenac was measurable at the first sampling time (0.33 h) in the majority of volunteers. The mean concentration-time profile of aceclofenac for the two formulations is shown in the Figure 1, which indicates that the mean plasma concentration profiles of the two brands were closely similar and superimposable. Peak concentrations of 8.64 ± 1.86 μg/ml and 9.36 ± 2.20 μg/ml for aceclofenac were attained at 1.99 and 1.91 h after administration of test and reference, respectively, and then declined rapidly but were detected up to 12 h.

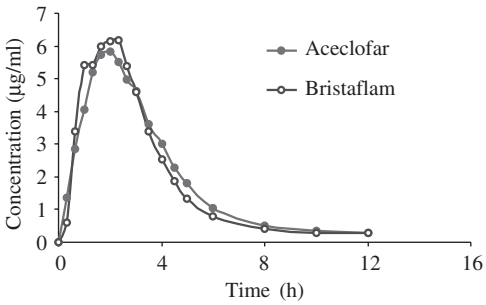

Figure 1. Mean plasma concentration of Aceclofenac 100 mg tablets after oral administration of single dose of two brands to 24 healthy human volunteers

Table 1. Pharmacokinetic parameters of aceclofenac tablets (mean ± standard deviation; $n = 24$)

Pharmacokinetic parameter	Aceclofar (test)	Bristaflam (reference)
AUC_{0-t} (μg/ml.h)	22.65 ± 4.48	21.88 ± 3.91
$AUC_{0-\infty}$ (μg/ml.h)	24.02 ± 4.74	23.17 ± 4.28
C_{max} (μg/ml)	8.64 ± 1.86	9.36 ± 2.20
T_{max} (h)	1.99 ± 0.80	1.91 ± 0.75
$T_{1/2}$ (h)	3.30 ± 0.68	3.36 ± 0.90
λ_Z (/h)	0.2207 ± 0.0560	0.2254 ± 0.0811

Table 1 shows the pharmacokinetic parameters of aceclofenac for the two brands. The relative bioavailability based on the geometric mean ratio was 1.032% for AUC_{0-t}, 1.034% for $AUC_{0-\infty}$ and 0.925% for C_{max}.

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent [19]. To demonstrate bioequivalence certain limits should be set depending on the nature of drug, patient population and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as *AUC* and C_{max}, the standard equivalence range is 0.8–1.25 [18]. The results of statistical analysis are shown in Table 2.

The mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two formulations did not differ significantly ($p > 0.05$), suggesting that

Table 2. Statistical analysis of log-transformed data

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (p-value)	0.0882 (0.1413)	0.0687 (0.0652)	0.2197 (0.3682)
90% CI	100.0–106.4% (99.7–105.8%)	100.2–106.8% (100.4–106.6%)	83.3–102.8% (95.2–117.6%)
Geometric mean ratio	1.032	1.034	0.925
Power of the study	1.0	1.0	0.88

Parentheses values indicate analysis for periods.

the plasma profiles generated by Aceclofar are comparable to those produced by Bristaflam. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, with p value greater than 0.05. Confidence intervals of 90% also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two formulations lie within the FDA acceptable range of 80%–125%.

For T_{max} the parametric point estimate of difference (test−reference) was 0.08 h, within the acceptance limits ($\pm$ 20% of reference mean).

Conclusion

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} clearly indicated no significant difference between Aceclofar and Bristaflam tablets in any of the calculated pharmacokinetic parameters. The confidence intervals for the ratios of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} indicated that these values were entirely within the bioequivalence acceptance range of 80%–125% (using log-transformed data). Based on the above it is concluded that Aceclofar, manufactured by Gulf Pharmaceutical Industries, UAE is bioequivalent to Bristaflam, manufactured by Bristol Myers Squibb, Egypt and that both products can be considered equally effective in medical practice.

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