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Research Article

A Comparative Study of the Pharmacokinetics of Conventional and Sustained-release Tablet Formulations of Aceclofenac in Healthy Male Subjects

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Abstract

Purpose: *To examine the pharmacokinetics of a formulated aceclofenac sustained release tablet formulation and determine if it is bioequivalent to a commercial brand of aceclofenac immediate release tablet (Zerodol® 100 mg).*

Methods: *Each of two groups of twelve fasting volunteers received either the reference standard (Zerodol 100 mg tablets) or the test formulation (200 mg aceclofenac) orally once, using a cross-over design with a one week wash-out period. Their blood samples were obtained at regular time intervals over 24 h and analyzed by high performance liquid chromatography (HPLC). Using the non-compartmental approach, plasma levels of aceclofenac were employed to compute their individual disposition kinetics, including peak plasma concentration (C_{max}), peak time (T_{max}), area under the plasma level-time curve (AUC_{0-t}), elimination rate constant (K_{el}) and elimination half life ($t_{1/2}$).*

Results: *The C_{max} values of 11043 ± 3073 ng/ml and 12301 ± 3000 ng /ml were attained in 2.58 ± 1.22 h and 1.29 ± 0.75 h for the test and reference products, respectively, while $AUC_{O-\infty}$ was 45996 ± 10427 and 50253 ± 8283 ng.h/ml, respectively. At 90% confidence interval, the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values of the test preparation were 96.4 - 101.3, 100.2 -101.9 and 98.5 - 99.8%, respectively, of the values for the reference. The $t_{1/2}$ values were found to be 4.50 ± 1.25 and 2.20 ± 2.59 h for the reference and test products.*

Conclusion: *On the basis of the pharmacokinetic data, it can be said that the test aceclofenac sustained release formulation and the reference product were bioequivalent in some respects. However, the test formulation exhibited a longer elimination half-life ($t_{1/2}$), thus demonstrating sustained release properties, unlike the reference.*

Keywords*: Pharmacokinetics; Bioequivalence; Sustained release aceclofenac formulation.*

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INTRODUCTION

Aceclofenac is a relatively new phenylacetic acid derivative showing marked anti-inflammatory, anti-arthritic, analgesic and anti-rheumatic activities [1]. The drug demonstrates better gastric tolerance than other non steroid anti-inflammatory drugs (NSAIDs) such as indomethacin and diclofenac [2]. Its recommended dose is 100 mg twice a day orally in the form of tablets. After oral administration, it is rapidly absorbed and bioavailability is almost 100% [3]. Peak plasma concentrations are reached within 1.25 to 3 h following ingestion [3]. It is highly protein–bound (> 99.7 %) and penetrates into the synovial fluid, where the concentration reaches up to approximately 60 % of that in plasma [3]. The volume of distribution is approximately 30 L while mean plasma elimination half-life and clearance are 4.3 ± 0.3 h and 5 L/h, respectively [3].

Aceclofenac is probably metabolized via Cytochrome P450 2C9 (CYP2C9, a protein encoded by the *CYP2C9* gene in humans) to the main metabolite 4-hydroxyacefenac with negligible contribution to clinical activity [3]. Its adverse reactions, as with other NSAIDs taken by mouth, are indigestion, heartburn, nausea and diarrheoa [3].

The objective of this study was to examine the *in vivo* bioequivalence of a test 200 mg aceclofenac sustained release formulation in relation to a commercial brand of 100 mg aceclofenac in male volunteers.

EXPERIMENTAL

Materials

The reference aceclofenac product used was an immediate release Zerodol® 100mg film-coated tablets, supplied (Ipca Laboratories, Mumbai, India). Aceclofenac was a gift from Mepro Pharmaceuticals Pvt. Ltd. Surendranagar, India. The excipients used in the production of the tablets were hydroxypropyl methylcellulose (HPMC) viscosity grade 4000 cps, (Methocel K4M, Colorcon Asia Pvt Ltd, Singapore) and lactose (DMV International, USA). Others were polyvinyl pyrollidone (PVP) K-30, (International Fine Chemicals Inc,, Canada), sodium propyl paraben (Salicylates and Chem Pvt Ltd, India), fumaric acid, magnesium stearate (Nitika Chemicals, India), talc (Udaipur Mineral Development Syndicate Pvt Ltd, India), colloidal silicon dioxide (Aerosil), isopropyl alcohol (Ranbaxy Fine Chemicals, India), methylene chloride (Chemplast Sanmar Ltd, India) titanium dioxide (Dupont Company Pvt Ltd, Singapore), PEG-6000 (Manali Petro Chemicals, India), castor oil (Sundarballi Oil Mill, India) and Ponceau 4 R supra (Roha Dye Chem, India). All other chemicals used were of analytical grade.

Preparation of aceclofenac sustained release tablets

The tablets were prepared by a wet granulation technique. The composition of the tablet formulations are given in Table 1. Aceclofenac, Methocel K4M, lactose/maize starch, sodium propyl para benzoate and fumaric acid were screened through a 425 µm sieve and mixed manually in a bowl for 5 min. The blend was granulated with the aid of PVP K-30 and water. The mass was sieved through a 500 µm sieve and then dried in a hot air oven at 50 ^{0}C. Magnesium stearate, talc and colloidal silicon dioxide were then added to the dried granules, mixed for about 5 min in a polythene bag and compressed into tablets using a 12- station tablet compression machine (CIP Machineries, Ahmadabad, India) equipped with a 11 mm biconcave-faced punches. To mask the bitter taste of the aceclofenac, the tablets were coated in a laboratory coater (Model GAC-250, Gansons Ltd, Mumbai, India) with HPMC 5cps dissolved in a mixture of isopropyl alcohol and methylene chloride; titanium dioxide and Ponceau 4 R supra (colouring agents) as well as polyethylene glycol (PEG) 6000 and castor oil (plasticizers) were also incorporated in the coating mixture prior to coating.

Table 1: Composition of sustained release tablet formulations

Ingredient	Weight (mg)
Aceclofenac	200
Methocel K4M	37.5
Colloidal silicon dioxide	4
Maize starch	33
Lactose	30
Purified water	q.s.
Povidone (PVPK-30)	7.5
Sodium propyl paraben	2
Fumaric Acid	10
Magnesium stearate	4
Talc	5

Compressing weight: 325 mg

Physiochemical characterization of the tablets

Tablet weight variation was evaluated on ten randomly selected tablets with an electronic balance (Mettler Toledo, Mettler, Griefensee, Switzerland) while tablet hardness and friability were also determined for ten randomly selected tablets using a Monsanto tablet hardness tester (standard type) and a Campbell electronic friabilator for 4 min at 25 rpm, respectively. Tablet diameter was evaluated with a digital vernier caliper.

Subjects and ethical issues

This study was performed in accordance with United States Food and Drugs Administration (FDA) Good Clinical Practice guidelines. Volunteers enrolled for this study were appraised in detail about all aspects of the study in easy to understand language and terminologies. Before admission to the study each subject was informed of the nature and the risks of the study and a written informed consent was obtained from the volunteers. The study involved twenty four healthy, adult, non-smoking male volunteers (mean age: 32.25 ± 5.53 years; mean height: 165.25 ± 3.96 cm; and mean weight: 62.25± 3.96 kg) with no revealed medical abnormality. The experimental protocol was approved by the instituional Central Ethics Committee (ref no. CEC/05/018) and the study was conducted at the Drug Monitoring Research Institute (DMRI), Sion, Mumbai, India.

Study design

All the volunteers were housed at the study centre. They were randomly divided into 2 groups of 12 subjects each. After an overnight fast (10 h), each group received orally, with 250 ml of water, either a single dose of the test sustained-release formulation or the reference product in a randomized fashion, using a two-treatment, two-way crossover study design. A seven-day washout period was allowed between dosing schedules.

Sample collection and handling

Blood samples (2ml) were collected by the intravenous route using heparinized disposable syringes at the following times: 0, 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 18, 20 and 24 hours after drug administration. The blood samples were collected in vacutainers containing EDTA as anticoagulant and immediately centrifuged at 3000 rpm for 15 min. The separated plasma samples were stored at -20^0 C until analysed.

Preparation of standard solutions

A stock solutions (1 mg/mL) of aceclofenac was prepared with methanol as the solvent. From the stock solution, standard solutions were prepared to contain 1, 2, 5, 10, 20, 30, 50 and 70 µg/mL of aceclofenac. A solution of ibuprofen (500 µg/mL) was prepared in a methanol/water mixture (80:20) and used as internal standard in the assay of aceclofenac.

Analysis of drug in plasma

To 100 µL of plasma, 25 µL of the internal standard and 200 µL of acetonitrile were added and mixed for a minute, and then made up to 1 ml with acetonitrile. The resulting solution was vortexed for 60 s and centrifuged at 10,000 rpm for 10 min. The supernatant layer was separated and

analyzed for aceclofenac content using a sensitive high performance liquid chromatographic (HPLC) method with a Shimadzu Class VP series incorprating Class VP 6.12 version software, two pumps (LC-10AT VP), a variable wavelength programmable UV detector (SPD-10A VP), a system controller (SCL-10A VP) and an RP C-18 column (Hypersil BDS C18). The operating conditions and parameters include: mobile phase: methanol + 0.3% tri ethyl acetic acid (60:40 v/v, pH 7.0); flow rate: 1.0 mL/min; injection volume: 20 mL; temperature: 25 ^{o}C; run time: 25 min; detection wavelength: 275 nm; and internal standard - ibuprofen. The response factor was measured by the ratio of the peak area of drug to that of the internal standard for both standard and test samples. Blank plasma samples were analyzed prior to the analysis of the standard and test preparations. Preliminary studies had shown that the presence of plasma did not interfere with the analysis of the drugs. The peaks were well resolved and the retention times were 5 min (aceclofenac) and 10.5 min (ibuprofen).

Data analysis

Using the non-compartmental approach, plasma levels of aceclofenac were employed to compute their individual disposition kinetics, viz, C_{max} (maximum plasma concentration), T_{max} (time to reach maximum plasma concentration), AUC_{0-t} (area under plasma concentration-time curve), $AUC_{0-\infty}$ (area under plasma concentration time curve, 0 to infinity), K_{el} (elimination rate constant) and $t_{1/2}$ (elimination half-life). Pharmacokinetic parameters were calculated with PK Solutions 2.0^{TM} Noncompartmental pharmacokinetic data analysis software.

Student's t-test was employed to analyze the results using Graph Pad Instat Software, version 1.13. Differences below the probability level of 0.05 were considered statistically significant. The ratios of the mean bioavailability parameters (test : standard) were obtained and 90 % confidence interval was used to determine bioequivalence. Log (natural) transformation of C_{max} and $AUC_{0-\infty}$ were performed prior to the statistical analysis. The equivalence criteria range was 80 to 125 % based on FDA guidelines. Calculations also included area under curve (AUC) from time t to ∞ (infinity) calculated with poly-exponential and trapezoidal methods.

RESULTS

Physicochemical properties of the formulated tablets

Tablet thickness was in the range 3.6 - 3.9 mm; diameter, 11.0mm; and hardness, 5.0 - 8.0 g/cm^2. Tablet friability and coefficient of weight variation (for individual tablets) were 0.5 and 1.4 to 3.5 %, respectively. Drug content (99 – 100 %) was uniform.

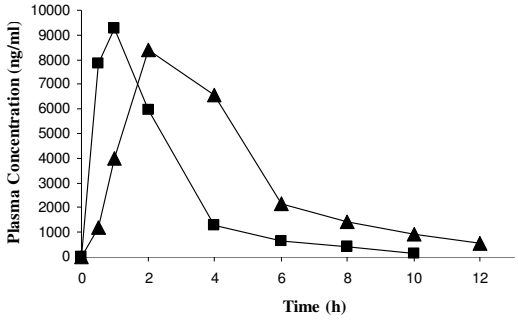

Figure 1: Mean plasma concentration versus time profile of test aceclofenac SR tablets 200 mg (▲) and reference standard tablets (■)

Pharmacokinetic profiles of aceclofenac tablets

Administration of the reference product as a single dose produced C_{max} of 12301 ± 3000 ng/ml at a t_{max} of 1.29 ± 0.75 h while C_{max} for the test preparation was 11043 ± 3073 ng/ml at a t_{max} of 2.58± 1.22 h (Table 2). The bioavailability of the test preparation relative to that of the standard was 91.1 %. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values of the test

preparation were 96.4 - 101.3 %, 100.2 - 101.9 % and 98.5 - 99.8 % of that of the reference, as shown in Table 3.

Table 2: Pharmacokinetic parameters for the test (A) and reference standard formulations (B)

Parameter	B (mean ± SD)	A (mean ± SD)
C_{max} (µg/ml)	12301±3000	11043±3073
t_{max} (h)	1.29±0.75	2.58±1.22
AUC_{0-t} (ng.h/ml)	47766±7891	43526± 10561
$AUC_{0-\infty}$ (ng.h/ml)	50253±8283	45997±10427
k_{el} (h^{-1})	0.427±0.245	0.238±0.183
$t_{½}$ (h)	2.20 ± 1.25	*4*.50 ± 2.59

Table 3: Bioavailability data for test aceclofenac formulation (200 mg) relative to the reference at 90% confidence interval

Parameter	Log transformed data
C_{max}	96.4 – 101.4 %
AUC_{0-t}	100.2 - 101.9 %
$AUC_{0-\infty}$	98.5 - 99.8 %

DISCUSSION

Aceclofenac was available in plasma within half an hour after its oral administration. The t_{max} of the test aceclofenac was significantly different($p < 0.05$) from that of the standard. Low t_{max} value for the reference drug (1.29 h) indicates rapid absorption while the higher t_{max} of the test drug (2.59 h) suggests slower absorption. This delayed absorption of test preparation is most likely due to the sustained release of the drug. On the other hand, the C_{max} of reference formulation was not significantly different from the test preparation. The half-life of the reference preparation was low which indicates rapid removal of the drug from plasma. This was also supported by the high elimination rate constant value. On the other hand, the test formulation exhibited higher half-life and low elimination rate constant values indicating slower drug disposition and prolonged effect. However, the $AUC_{0-\infty}$ values for the two formulations were not significantly different. There was also no difference in the extent of absorption for both the test and reference products as indicated by the less than 10 % difference in relative bioavailability (91.1 versus 100 %) of the drug. This suggests that the aceclofenac contained in the test product was completely absorbed.

CONCLUSION

The test product showed sustained release characteristics unlike the reference. Overall, the pharmacokinetic profile of the former was either equivalent or superior to that of the reference. Thus, the test aceclofenac preparation can be further developed to achieve a suitable sustained release product.

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