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RESEARCH ARTICLE

BIOEQUIVALENCE STUDY OF ETODOLAC ER TABLETS 600MG UNDER FASTING CONDITION

Mathew George[1]*., Lincy Joseph[2]., Viyas[3] and Prabha Mathews[2]

[1]Department of Pharmacology, Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India
[2]Department of Pharmaceutical Chemistry, Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India
[3]Department of Pharmacology, School of Pharmaceutical sciences, Jaipur National University, Jaipur-302050, Rajasthan

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ABSTRACT

Aim: Bioequivalence study of etodolac ER tablets 600mg under fasting condition.
METHOD: Normal healthy adult male human subjects received either 600mg of the reference or test formulation in fasting (N=12) condition. The study was conducted according to a single dose and randomized crossover design. Blood samples were collected upto 48.00hours after drug administration. Plasma concentrations of Etodolac were determined by LC-MS/MS. Pharmacokinetic parameters were calculated from the observed plasma concentration- time profiles. Bioequivalence between the formulations were found out considering 90% confidence interval for the ratio of means for C_{max}, AUC_{0-inf} and AUC_{0-t} within 80-125%.

Results: The 90% confidence interval for the test, the ratio of the means for C_{max} (92.09-119.66), AUC_{0-inf} (98.22-117.6) and AUC_{0-t} (92.70-110.21) and was within the guidelines range of bioequivalence (80-125%).

Conclusion: So based on results we can conclude that the pilot study of Etodolac Tablet was performed with high accuracy with compliance of all the regulatory requirements. Based on the statistical analysis test products of Etodolac ER tablets 600mg of sponsor's formulation is bioequivalent to Reference product Etodolac ER tablets 600mg in terms of rate and extent under fasting condition. So, the formulation of the test product is passing the study and with the higher sample size in the pivotal study will also give positive results.

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INTRODUCTION

Life expectancy of patients has increased globally during the last three decades due to the new drug discovery (brand-name drugs) as well as generic drug production. The rising cost of medication has been contributing to the total overall cost of health care and thus receives considerable attention globally. A major strategy for lowering the cost of medication and thereby reducing its contribution to total health care costs, has been the introduction of generic equivalents of brand-name drugs (innovator drugs) (Midhal KK *et al*, 2009). The increased availability and use of generic drug products, healthcare professionals are encountered with a large number of multisource products from which they have to select therapeutically equivalent products. Generic substitution is of concern not only for healthcare professionals but also for pharmaceutical industries, consumers and government officials. Many research papers have pointed out the concern regarding standards for approval of generic products which may not always ensure therapeutic equivalence (Boix-Montanes A , 2011: Skelly JP , 2010; Tothfalusi *et al*, 2009; Midha *et al*,2005; Chen ML *et al*,2001; Chen ML *et al*,2000; Strom BL 1987; Lamy PP , 1986). Many guidelines/guidance and regulations covering the licensing of generic products have been introduced to ensure that the medicinal products reaching the market have well-established efficacy and safety profile (FDA, 2003; FDA, 2011; CDSCO, 2005).

Generic drugs have captured more than 65% of the global market and account for 66% of prescriptions filled in the United States but for less than 13% of the cost. Thus, because of the importance of generic drugs in health care, it is imperative that the pharmaceutical quality, safety, and efficacy of generics should be reliably compared with the corresponding innovator drugs (brand-name drugs). The US Food and Drug Administration (FDA) publishes a list of drug products and equivalents, approved drug products with therapeutic equivalence evaluations, commonly known as the

—Orange Book.(Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations 2010)

Generic pharmaceutical products need to conform the same standards of quality, safety and efficacy of the originator's product. In addition, they should be clinically interchangeable with equivalent marketed products. To ensure interchangeability, the generic product must be therapeutically equivalent to the reference product. Therapeutic equivalence can be assured when the generic product is both pharmaceutically equivalent/alternative and bioequivalent (CEBS, 2000) The efficacy and safety of medicinal products should be demonstrated by clinical trials which follow the guidance in 'Good Clinical Practice: Consolidated Guideline' (ICH E6) adopted by the ICH, 1 may 1996. In BE studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product. For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit the same rate and extent of absorption as the reference drug product. Manufacturers seeking regulatory approval of competitive (generic) products (e.g. Abbreviated New Drug Application [ANDA]), must provide detailed bioavailability evidence showing head-to-head comparative performance of their product against the innovator's product. Selected pharmacokinetic parameters and preset acceptance limits allow the final decision on bioequivalence of the tested products. AUC, the area under the concentration time curve, reflects the extent of exposure. Cmax, the maximum plasma concentration or peak exposure, and the time to maximum plasma concentration, tmax, are parameters that are influenced by absorption rate.(Rasma Chereson, 1997)

Etodolac is a synthetic analgesic. It inhibits both COX-1 and COX-2(Limbard L,2006). But has more selectivity for COX-2. Post marketing surveillance studies suggest that Etodolac has a low risk of stomac ulceration and internal bleeding. So its use is beneficial compared to other NSAIDs. Etodolac is indicated for the management of minor to moderate pain in adults. It is a choice of drug in rheumatoid arthritis. Etodolac 300 to 600 mg can be administered as needed for pain relief every 4 to 6 hours not to exceed 800mg/day. FDA's approved drug products with therapeutic equivalence evaluations (Orange book) suggest any test formulation of Etodolac ER tablets 600mg is therapeutic equivalent to Etodolac ER tablets 600mg. So reference formulation was selected. Reference listed drug (RLD) label claim suggest that food does not significantly interfere with the rate or extent of absorption of Etodolac, therefore, Etodolac can be administered without regard of food. But here sponsor's attempt is to approve generic version which demonstrates different pharmaceutical properties from reference listed drug (RLD). So attempt has been focused to conduct fasting bioequivalence study.

MATERIAL AND METHODS

A randomized, open label, balanced, two-treatment, two period, two sequence, single dose, crossover design study of Etodolac ER tablets 600mg under fasting condition was conducted in compliance with the current version of the declaration of Helsinki, the current ICH GCP Guideline, USFDA, GLP and relevant Natinal Laws and Regulations and fulfilled the objectives of the pilot study.

Study protocol was designed based on the recommended guidelines, drug label and literature survey. Study protocol was approved by the sponsor and the independent ethics committee. The study was conducted in accordance with this protocol. No protocol amendment or major deviation was taken from all the subjects at the time of screening.

All participants signed a written informed consent after they had been informed of the nature and details of the study. Volunteers were screened before the study. The screening procedures included demographic data, clinical history, physical examination (including vital signs), haemogram, biochemistry and urine analysis. All the evaluation parameters were found within the normal clinically acceptable range.

Subjects excluded were with hypersensitivity to study medications or related products, significant history of psychiatric, gastrointestinal, liver or kidney disorder/impairments, or any other conditions known to interfere with the absorption, distribution, metabolism or excretion of common medications, significant history of asthma, chronic bronchitis or other bronchospastic condition, significant history or presence of glaucoma, cardiovascular or hematological disease or diabetes or metabolic acidosis or with a known food allergy, any clinically significant illness during the 4 weeks prior to day one of this study or hospitalized during 3 months prior to the commencement of this study, maintenance therapy with any drug, or history of drug dependency, alcohol abuse, or serious neurological or psychological disease, participation in a clinical trial with an investigation drug within 90 days preceding day 1 of the current study, use of enzyme-modifying drugs within 30 days prior to day 1 of this study, use of any systemic medication (including OTC preparations) within 14 days preceding day 1 of this study, HIV and Hepatitis positive findings.

Volunteers were arrived at Pharmacokinetic Unit, Bio Evaluation Centre at least 12hours before dosing and confined for 24 hours after drug administration in each period. A standard dinner was served to the subjects at least 10 h before dosing. Single oral dose (1×600mg tablet) of either test product A or reference product B was administered as per randomized schedule in each period with 240ml of water at ambient temperature in sitting position. Blood sample collection was done using IV cannula. The IV cannula was inserted into the subject's arm for collection of blood samples before pre-dose blood sample and for upto 48.00hrs post dose. Post-dose sampling times after formulation administration were 0.33, 0.67, 1.0, 1.33, 1.67, 2.00, 2.33, 2.67, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0 and an ambulatory sample at 48.00 h. A total of 20 blood samples (5ml each) were collected from each subject in each study period. Blood samples were centrifuged for 10 minutes at 3500rpm at 5^0 C . Plasma was separated and stored frozen at –70 °C ± 10°C with appropriate labels for identification. A standard lunch was served to subjects, at least 4 h after dosing. Food and time of feeding were identical in all periods of study.

The study was planned and conducted in 12 subjects aged 18-45 years. No subject was withdrawned or dropped out from the study during any study period. A total number of 12 subjects completed the clinical phase of the study. Hence the plasma samples of these 12 subjects were analyzed and considered for drawing conclusion. There was no adverse event report during the study.

The analysis of Etodolac in plasma samples was performed by validated LC-MS/MS methodology which allowed specific and sensitive determination of Etodolac in plasma. A total of

12 subjects data was taken for statistical analysis. Samples were separated by adding all frozen plasma samples and vortexing each plasma sample for about 30 seconds and centrifuging at 14000rpm at 10^0C for 5 minutes. Blank human plasma was obtained for healthy volunteers. Zero standard was prepared by mixing 0.1ml blank 50µl IS-2(500µg/ml) and 0.1ml 5% (v/v) orthophosphoric acid. Calibration standard and quality control samples were prepared by mixing 1.0ml of plasma samples containing known concentration of analyte, 50µl IS-2 and nd 0.1ml 5% (v/v) orthophosphoric acid in water. All the subject samples were then vortexed for 30 seconds followed by centrifugation at 14000rpm for 5 minutes at 10^0C.

Solid phase extraction was used as extraction procedure. 30mg/ml were separated for quality control samples & subject samples. After the removal of interferences by washing the catridges with 1ml (10%v/v) methanol in water followed by 1ml water, the analyte was eluted with 2ml mobile phase. Indomethacin was used as internal standard (IS). 1µl volume was injected into LC/MS/MS system. Acetonitrile (20:80v/v) was used as mobile phase. 2mM ammonium acetate in water was used as buffer. Column of C18,(50mmX4.6mm), 1µl was used and maintained at 30^0C. Flow rate was adjusted to 0.3ml/min. methanol: water in the ratios (20:80)& (50:50) was used as diluents. Mass to charge ratio (m/z) for Etodolac, parent ion was 286.3 amu, product ion 212.1 amu. And for indomethacin parent ion 356.4amu, product ion 312.0amu Plasma concentrations were presented with mean, standard deviation and percentage coefficient of variation for each sampling time point for both the formulations of Etodolac. Descriptive statistical analysis were presented for all primary (C_{max}, AUC0-t, AUC0-inf) and secondary , AUC_{0-t} / AUC_{0-inf} , T_{max}, K_{el} and $t_{1/2}$) pharmacokinetic parameters.

RESULT

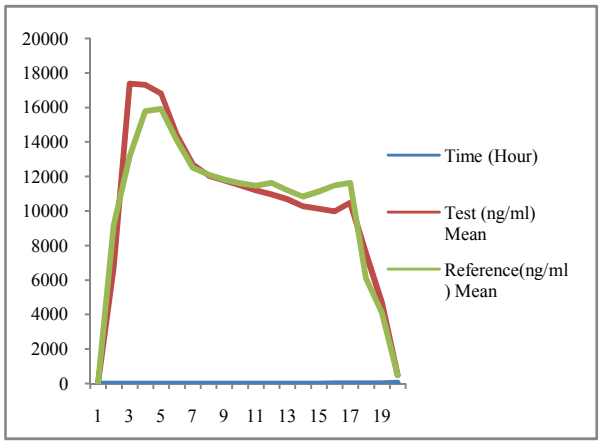

Figure 1 Time vs Plasma concentration graph

Table 2 pharmacokinetic parameters

Pharmacokinetic parameters (N=12)	Test		Reference	
	Mean	±SD	Mean	±SD
C_{max}(ng/ml)	18650.92	4836.444	17583.3	3908.569
$AUC_{(0-t)}$(ng×hr/ml)	260928.6	119858.7	254269.8	107126.1
$AUC_{(0-inf)}$(ng×hr/ml)	304631.9	113301.9	284534.8	111984.3
T_{max}(hrs)	2.916667	0.792961	3.75	2.767506
K_{el} (hrs/l)	0.069501	0.019298	0.087478	0.02656
$T_{1/2}$ (hrs)	10.74082	3.189739	8.655865	2.782623

Statistical Analysis

Table 3 90% confidence interval based on geometric means of Log transformed PK parameters for test product (A) and reference product (B)

Parameters	*Geometric mean		% Ratio A/B	90% Confidence Interval for Log transformed data	
	Test (A)	Reference (B)		Lower limit	Upper limit
$AUC_{(0-inf)}$ (ng×hr/ml)	288311.39	268406.05	107.41	98.22	117.46
$AUC_{(0-t)}$ (ng×hr/ml)	24050.92	237928.69	101.08	92.70	110.21
C_{max}(ng/ml)	18077.16	17219.56	104.9	92.09	119.66

*geometric mean has been taken as the antilog (exponential) of the least square mean of the log transformed data.

Table 1mean Plasma Concentrations (ng/ml) for test and reference formulation

Time (Hour)	Test			Reference		
	Mean	SD	CV%	Mean	SD	CV%
0.00	0.000	0.000	-	0.000	0.000	-
0.33	6712.383	5819.571	86.699	9176.804	3535.237	38.524
0.67	17375.278	4704.379	27.075	13164.691	4422.656	33.595
1.00	17301.263	4704.379	27.075	15769.506	5104.922	32.372
1.33	16807.787	4997.950	29.736	15906.005	5764.300	36.240
1.67	14421.270	4875.797	33.810	14098.205	4651.83	32.996
2.00	12697.818	4278.271	33.693	12492.707	3783.365	30.285
2.33	12049.888	4041.333	33.538	12103.092	3484.257	28.788
2.67	11768.825	3926.025	33.360	11817.848	3491.470	29.544
3.00	11488.909	3940.581	34.299	11600.503	3486.719	30.057
4.00	11189.896	3713.160	33.183	11448.571	3689.057	32.223
5.00	10954.017	3583.316	32.712	11624.617	3464.865	29.806
6.00	10679.029	3469.345	32.487	11196.779	3098.022	27.669
7.00	10271.514	3417.488	33.272	10834.091	3194.273	29.484
8.00	10123.382	3777.961	37.319	11124.708	3295.910	29.627
10.00	9968.433	3433.660	34.319	11482.427	2856.329	24.876
12.00	10474.093	3427.552	32.724	11627.930	3310.554	28.471
16.00	7547.151	2913.014	38.598	691.802	2355.759	33.741
24.00	4672.257	2239.407	47.93	4091.936	2097.963	51.271
48.00	503.899	802.181	159.195	485.566	849.032	174.854

Table 4 Anova Calculation

	C_{max}	$AUC_{(0-t)}$	$AUC_{(0-inf)}$
	ANOVA p-value		
Sequence	0.3537	0.2323	0.3211
Period	0.9969	0.1692	0.3339
Treatment	0.5162	0.8260	0.1777

DISCUSSION

Results for mean T_{max} values for test –A (3.00 hrs) and for reference – B (3.00hrs) show that the time to reach the peak concentration is lower for test product as compared to the reference product indicating good release of drug from test formulation as compared to the reference formulation. The mean k_{el} values for test – A (0.07±0.02hrs^{-1}) and for reference – B (0.09± 0.03hrs^{-1}) were almost same for both the formulations. The mean half life values for test – A (10.47±3.19hrs) and for reference – B (8.66±2.78hrs) were almost same for both the formulations. The untransformed mean C_{max} value for test – A (18650.92±4836.44ng/ml), and for reference – B (17583.30±3908.57) showed no significant difference among the formulations. The 90% confidence interval for the log transformed ratio of means for the test – A ftor C_{max} (92.09 – 119.66) $AUC_{0\text{-}inf}$ (98.22-117.46) and $AUC_{0\text{-}t}$ (92.70-110.21) and is within the bioequivalence range (80-125%). It indicates that the test formulation is bioequivalent to the reference drug.

There were no adverse effects reported during the study. It can be concluded that the formulation of Etodolac was well tolerated by healthy subjects, as a single dose administration and no relevant differences in the safety profiles of the test and reference formulations were observed.

CONCLUSION

In this study plasma concentrations of Etodolac were measured by validated LC-MS/MS analytical method. Individual and mean plasma concentrations of 12 subjects were utilized for pharmacokinetic and statistical analysis at different sampling time points of reference and test of Etodolac. We performed statistical analysis for all primary and secondary ($C_{max,}$ $AUC_{0\text{-}inf,}$ $AUC_{0\text{-}t,}$ $AUC_{0\text{-}t}$/ $AUC_{0\text{-}inf,}$ $T_{max,}$ $k_{el,}$ $t_{1/2}$)pharmacokinetic parameters.

The 90% confidence interval for the test, the ratio of the means of $C_{max,}$ (92.09 – 119.66) $AUC_{0\text{-}inf}$ (98.22-117.46) and $AUC_{0\text{-}t}$ (92.70-110.21) and is within the bioequivalence range (80-125%).

So based on results we can conclude that the pilot study of Etodolac tablets was performed wit high accuracy and with compliance of all the regulatory requirements. Based on the statistical analysis test products of Etodolac ER tablets 600mg of sponser's formulation is bioequivalent to reference product Etodolac ER tablets 600mg in terms of rate and extent under fasting condition.

So the formulation of test product is passing the study. Therefore when study is conducted with higher sample size, the result will also be positive.

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