A Randomized, Crossover Study to Determine Bioequivalence of S-Etodolac ER Tablets Versus Etodolac ER Tablets in Healthy Indian Subjects

Menon S
Kadam N
Gursale A
Gokarn V
Palekar A

1Therapeutic Drug Monitoring Laboratory
Mumbai, India

ABSTRACT

Objective

To estimate the bioavailability and evaluate bioequivalence of S-Etodolac 300 mg tablet and to compare it with that of Etodolac 600 mg tablet

Materials and Methods

Using a two-treatment, two-period, two-sequence, randomized crossover design, test and reference formulations were administered as individual single doses to 24 healthy adult Asian male subjects of Indian origin under non-fed conditions, with one week washout period between dosing. Pharmacokinetic parameters, Cmax, AUC0-t, AUC0-∞ and Cmax/AUC0-∞ were calculated from the plasma concentration-time data of each individual and during each period by applying non-compartmental analysis. Analysis of variance was carried out using logarithmically transformed and non-transformed values of the stated pharmacokinetic parameters. Data for test and reference formulations were analyzed statistically to test for bioequivalence of the two formulations.

Results and Discussion

All subjects completed the study. After oral administration the mean values of Cmax (µg/ml), AUC0-t (µg/ml*h), and AUC0-∞ (µg/ml*h) for reference and test formulations were 3.94 and 4.07, 23.02 and 23.20, 27.49 and 28.42, respectively. ANOVA and CI test showed no significant (p > 0.05) variation in these pharmacokinetic parameters of test and reference formulations. The T/R ratio of geometric mean of Cmax, AUC0-t and AUC0-∞ for S-etodolac in both formulations was 106.94%, 103.93% and 105.64% respectively. These values are within the acceptance limit of 80 – 120%. No adverse events or clinically significant changes were observed in any of the subjects during the two runs of the study.

Conclusion

The test formulation containing S-Etodolac 300 mg was bioequivalent to S-Etodolac from reference formulation (Etodolac 600 mg).

INTRODUCTION

Many drugs have been developed as a racemic mixture (50:50) of the S- and R-enantiomers. Etodolac is a Nonsteroidal anti-inflammatory drug (NSAID) of 2-arylpropionate class is an important group of racemic medication. The S-isomer of NSAIDs is generally thought to express

pharmacological activity and/or be associated with clinical efficacy [Evans, 1992., Liang and Hsu, 2003]. S-(+)-Etodolac shows almost all the pharmacological activity, while R-(-)-etodolac shows little [Demerson et al, 2003]. S-Etodolac is 2.6 times more potent than the racemate. S-Etodolac possess almost all of the anti-inflammatory activity of etodolac while R-Etodolac is almost inactive [Demerson et al, 2003]. It is indicated for the management of pain, and for the management of the signs and symptoms of rheumatoid arthritis and osteoarthritis. Etodolac is an NSAID which is more selective for induced COX-2 (associated with inflammation) over COX-1 (cytoprotective) [Shi and Li, 2000., Demerson et al, 2003]. As a COX-2 selective inhibitor, etodolac can not only enhance its anti-inflammatory and analgesic activity but also improve patient compliance [Demerson et al, 2003].

Etodolac has been found to show stereoselective pharmacokinetics in human and in the rat [Jamali et al, 1988., Demerson et al, 2003]. S-Etodolac rapidly attains the peak plasma concentration and is rapidly cleared from plasma compared to R-Etodolac. The pharmacologically inactive R-Etodolac has higher plasma concentration compared to S-Etodolac [Shi et al, 2004., Brocks and Jamali, 1990]. S-Etodolac attains greater concentrations in synovial fluid than plasma compared to R-Etodolac [Brocks et al, 1991]. The pharmacokinetic differences are attributed to the greater extent of plasma protein binding of R-Etodolac, and to preferential conjugation and biliary excretion of S-Etodolac [Shi et al, 2004., Brocks and Jamali, 1990]. In addition, findings from human serum albumin (HSA) study suggest that R- and S-Etodolac interact mainly with site II of HSA and are displaced by each other [Mignot et al, 1996]. Moreover, etodolac enantiomers may show different pharmacokinetic characters when given individually. So our aim was to study the bioavailability and evaluate bioequivalence of a single dose of a enantiomer S-Etodolac tablet (test formulation, containing S-Etodolac 300 mg, manufactured by Emcure Pharmaceuticals Ltd., Pune, India) and compare it with that of a single dose of Etodolac tablet (reference formulation, containing Etodolac 600 mg, manufactured by Teva Pharmaceuticals, USA) under fasting conditions was given orally.

MATERIALS AND METHODS

Test and Reference formulations

Test medication: S-Etodolac 300 mg extended release tablets manufactured by Emcure Pharmaceuticals Limited, Pune, India, Batch No. FD/1580/07

Reference medication: Etodolac 600 mg extended release tablets manufactured by Teva Pharmaceuticals USA, Batch No. 263060

Subjects and Methods

This study was conducted at the Therapeutic Drug Monitoring Laboratory, Mumbai, India, according to Good Clinical Practice in compliance with the ethical norms laid down in the guidelines issued by the Indian Council of Medical Research, New Delhi, 2000, and the Declaration of Helsinki, 2004, Tokyo. This study was conducted with a due permission from Drug Controller General of India (DCGI) and after the study protocol was approved by the Institutional Ethical Review Committee of the study center. All participants signed a written informed consent after they had been informed of the nature and details of the study.

This was an open label, comparative, randomized, single dose, cross-over study with a wash-out period of one week between dosing. An equal number of subjects were randomly assigned to each dosing sequence of treatments. In a randomized, crossover and comparative design, 24 healthy adult, non-smokers, Asian male volunteers of Indian origin, with mean ± SE age and weight of 26.8 ± 1.20 years and 59.6 ± 1.40 kg, respectively, were included. 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 proceeding day 1 of this study, HIV and Hepatitis positive findings.

Subjects were confined at the clinical site at least 13 h before and till 24 h after dose administration. A standard dinner was served to the subjects at least 10 h before dosing. In the morning of dosing, an indwelling Teflon needle was introduced in the left/right forearm vein and pre-dose blood sample was collected in centrifuge tubes containing 0.1 ml of 10% EDTA. The drug was administered orally with 240 ml of water after an overnight fasting. Post-dose sampling times after formulation administration were 1.00, 2.00, 3.00, 4.00, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 9.00, 10.00, 12.00, 18.00, 24.00 and an ambulatory sample at 48.00 h. Blood samples were centrifuged for 10 minutes at 4000 rpm (1500 G). The centrifugation was done within 10 minutes of blood collection. Plasma was separated and stored frozen at –20 °C ± 5 °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. No

water was permitted 1 h before and 2 h after dosing. Water was allowed ad libitum 2 h after dosing. Subjects abstained from alcohol for each study run and no consumption of alcohol was permitted from 48 h prior to dose administration till the end of follow-up examination. Xanthine-containing products were not allowed 48 h before dosing and until the end of each cycle period. Throughout the duration of the study the subjects were closely monitored for evidence of study formulation intolerance and for development of clinical or laboratory evidence of adverse events. Emergence of symptoms, if any, was also noted by the subjects at the end of the study in the symptom checklist form. Subjects were housed in the clinical investigation unit for 24 h under the supervision of clinical attendants.

HPLC assay of S-enantiomer in plasma

S-(+)-etodolac concentrations were measured using the HPLC method developed and validated at the analytical facility for the enantiomer specific quantification of etodolac. The analytical method was validated prior to the start of the study. The validation parameters included specificity, ruggedness, LOD and LOQ, calibration curve, precision, accuracy, recovery, quality control samples and stability. The HPLC system consisted of, the Jasco HPLC PU 980 pump fitted with AS-1555-10 auto sampler, Jasco UV-970, UV-Visible and Chiralcel OD column (25 cm X 0.46 cm i.d.). The mobile phase contained n-Hexane: 2-Propanol in the volume ratio 85:15 + 0.05% Trifluoroacetic acid. 200 µl of drug-free plasma was taken in tubes. To these standard solutions were spiked to obtain the concentrations of 1 µg/ml, 2 µg/ml, 8 µg/ml, 22 µg/ml, 30 µg/ml, 50 µg/ml and 80 µg/ml, respectively. The tubes were vortexed for 30 s. To this 0.08 ml of 1M HCL was added and the tubes were vortexed for 30 s. To this 4 ml of 5% Ethyl Acetate in n-Hexane was added and the tubes were vortexed for 30 seconds. The tubes were then centrifuged for 10 min at 2000 rpm. 3.5 ml of organic layer was collected and evaporated at 70 °C under a

Table 1. *Geometric mean for S- Etodolac ER tablets versus Etodolac ER tablets*

	Reference Formulation	Test Formulation	T/R Ratio (%)
Cmax (µg/ml)	3.70	3.95	106.94
AUC (0-t) (µg.hr./ml)	21.20	22.03	103.93
AUC (0-∞) (µg.hr./ml)	25.43	26.86	105.64

Table 2. *90% confidence interval for the pharmacokinetic parameters of S-Etodolac ER tablet versus Etodolac ER tablet*

Parameters	Lower limit (%)	Upper limit (%)
Cmax	94.64	111.94
AUC (0-t)	90.66	110.87
AUC (0-∞)	89.82	116.96
lnCmax	98.48	116.12
lnAUC (0-t)	94.95	113.73
lnAUC (0-∞)	94.72	117.81

stream of nitrogen for 10 min in a low volume evaporator. The residue was then reconstituted in 200 µl of the mobile phase, and 20 µl of the reconstituted residue was injected into the HPLC system. Stability of S-Etodolac in plasma was evaluated as freeze-thaw stability, bench top (room temperature) stability, short term stability (6 h) and long term stability (30 days). In addition, short- (6 h) and long-term stock (14 days) solution stability and HPLC autosampler stability were also evaluated. The acceptance criteria for the validated method were based on precision, accuracy, sensitivity, specificity and stability.

Pharmacokinetic analysis

The plasma levels produced by the administration of the test and reference formulations in each subject were used to establish the pharmacokinetic profile of all formulations. Apparent first-order elimination or terminal rate constant was calculated from the semi-log plot of the plasma concentration versus time curve. This parameter was calculated by the linear least square regression analysis using the last three (or more) non-zero plasma concentrations. The elimination $t1/2$ is obtained by dividing 0.693 by elimination rate constant (Kel); tmax and Cmax were observed values. For all participants, the AUC profiles were determined by using the trapezoidal rule during the ascending portion of the curve and the log-trapezoidal rule during the descending portion of the curve. Samples with a concentration below the LOQ were assigned a value of zero. The AUC values for each participant and treatment were evaluated over the time intervals of 0 (time of dose) to infinity. It is assumed that the racemate etodolac (reference formulation in this study) have 1:1 ratio of both enantiomers. This assumption was predicated on the inability of etodolac enantiomers to interconvert due to the presence of its chiral centre within an alicyclic ring. The concentrations of etodolac in plasma and derived pharmacokinetic parameters among treatments were compared by using an analysis of variance (ANOVA). In the analysis the effects of sequence, subject nested within sequence, period and treatment were evaluated. Statistical differences at a probability of $p < 0.05$ were considered significant. The pharmacokinetic parameters were further analysed by Scheffe's method testing to discern where specific differences between treatments existed. In examining the mean concentration profiles, the reference was chosen as that which most resembled the Cmax, tmax, and AUC of etodolac. The ratio of test to reference least-squares geometric means of Cmax , AUC0-t, and AUC0-∞ and the corresponding 90% confidence limits were calculated by using the mean square error from the 2-period crossover ANOVA and were performed on logarithmically transformed data. The results for geometric mean are tabulated in Table. 1. The upper and lower limits of the 90% confidence interval (CI) were then antilogarith-

Table 3. *Pharmacokinetic parameters for S- Etodolac ER tablets versus Etodolac ER tablets*

	Reference Formulation			Test Formulation		
Parameters	Mean ± S.D.	S.E.	% CV	Mean ± S.D.	S.E.	% CV
Cmax (µg/ml)	3.94 ± 1.40	0.29	4.04	4.07 ± 0.99	0.20	24.29
AUC (0-t) (µg. hr./ml)	23.02 ± 9.92	2.03	22.27	23.20 ± 7.49	1.53	32.30
AUC (0-∞) (µg. hr./ml)	27.49 ± 11.95	2.44	24.38	28.42 ± 10.05	2.05	35.38
AUC (0-t)/ AUC (0-∞) (%)	85.44 ± 16.55	3.38	19.37	84.69 ± 18.02	3.68	21.28
Cmax/AUC (0-∞)	0.15 ± 0.05	0.01	0.14	0.15 ± 0.04	0.01	26.07
lnCmax (µg/ml)	1.31 ± 0.37	0.08	1.39	1.37 ± 0.25	0.05	18.30
lnAUC (0-t) (µg. hr./ml)	3.05 ± 0.41	0.08	3.10	3.09 ± 0.33	0.07	10.76
lnAUC (0-∞) (µg. hr./ml)	3.24 ± 0.40	0.08	3.19	3.29 ± 0.34	0.07	10.38
lnCmax/AUC (0-∞)	-1.93 ± 0.31	0.06	-1.95	-1.92 ± 0.27	0.05	-13.96

Table 4. *Back calculated concentrations of calibrant samples for S-Etodolac (between-run)*

		Calibrant Samples						
		1.00 µg/ml	2.00 µg/ml	8.00 µg/ml	22.00 µg/ml	30.00 µg/ml	50.00 µg/ml	80.00 µg/ml
Day 2	Linearity 1	1.06	1.96	7.86	22.54	29.06	47.22	83.29
Day 3	Linearity 2	1.10	2.02	6.91	22.02	31.36	47.84	81.74
Day 4	Linearity 3	1.11	2.05	7.13	22.86	29.08	43.69	87.08
Mean		1.09	2.01	7.30	22.47	29.84	46.25	84.04
SD		0.03	0.04	0.49	0.42	1.32	2.24	2.75
% CV		2.66	2.21	6.77	1.87	4.43	4.84	3.27
% Nominal		109.18	100.41	91.25	102.16	99.45	92.51	105.05

Table 5. *Curve parameter summary for S-Etodolac (between-run)*

	Curve code	Slope (a)	y-intercept (b)	Coefficient of Determination (r2)
Day 2	Linearity 1	13997.76	931.96	0.9986
Day 3	Linearity 2	15707.42	813.37	0.9964
Day 4	Linearity 3	15024.78	-4073.24	0.9915
Mean		14909.98		0.9955
SD		860.59	Not applicable	0.004
% CV		5.77		0.37

Figure 1. *Plasma concentration (in µg/ml)-time (in hours) profile of S-Etodolac after single dose oral administration of reference and test formulations in 24 healthy volunteers*
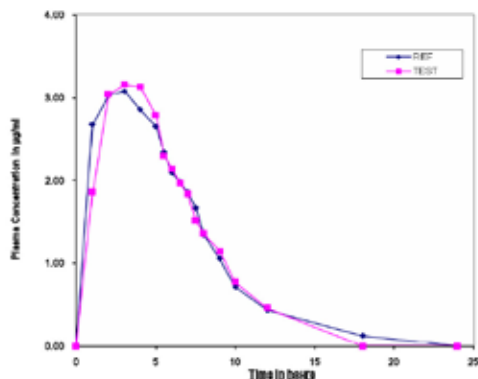

Figure 2. *Plasma concentration of reference (in µg/ml)-time (in hours) profile of S-Etodolac after single dose oral administration in 24 healthy volunteers*

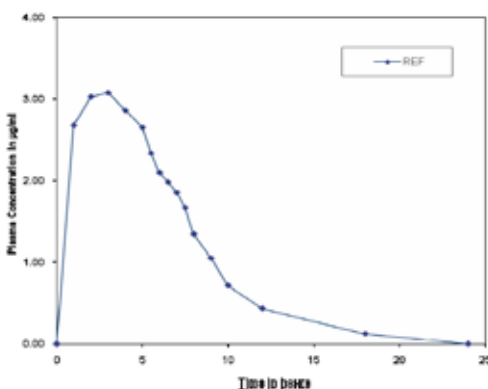**Figure 3**. *Plasma concentration of test (in µg/ml)-time (in hours) profile of S-Etodolac after single dose oral administration in 24 healthy volunteers*
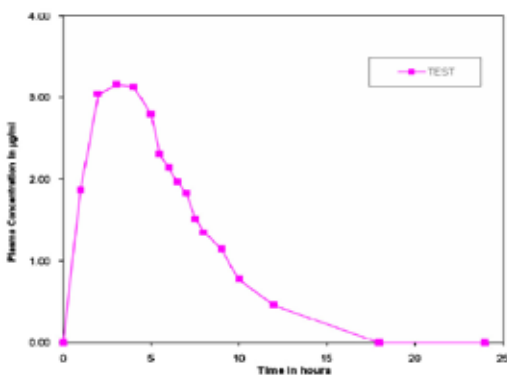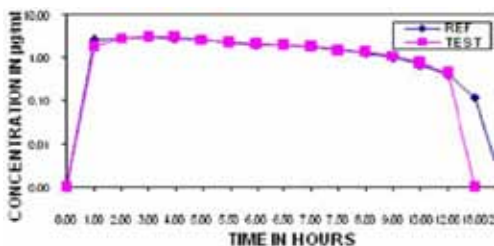

Figure 4. *Semilog plot of plasma concentration of S-Etodolac ER tablet versus Etodolac ER tablet*

mically transformed to the linear scale. Two one-sided t-tests bioequivalence procedure for log-transformed and untransformed data was also applied to these data. For logarithmically transformed data, this test procedure is equivalent to requiring the ordinary 90% CI of the geometric mean ratio to lie within 80 to 125%. It is shown in Table. 2.

To examine the impact of rate of drug absorption on the bioequivalence parameters, a plot of the ration of C_{max} least-squares geometric mean versus t_{max} for each regimen was plotted for its respective analyte. Conformity of these point estimates and their 90% CIs within the 80 to 125% bound of the ratio of least-squares geomet-

ric mean dictates whether a test treatment would be considered bioequivalent to the reference. The parameters were derived individually for each subject from the S-Etodolac concentration in plasma. The actual times of blood sampling were used for this calculation. Non-compartmental methods were used. Arithmetic means, standard deviations, standard error and coefficient of variation for all parameters were calculated. The results are shown in Table.3. Pharmacokinetic differences due to race have not been identified. Clinical studies reported have included patients of many races, all of whom responded in a similar fashion.

Statistical analysis of data

Standard descriptive analysis including mean, standard deviation (SD) and standard error (SE) are used for variables such as the height, weight and age. Along with these

statistical parameters, coefficient of variance (CV) was used to describe plasma concentrations at each time, untransformed and log-transformed (Cmax, AUC0-t, AUC0-∞) pharmacokinetic parameters. Untransformed and log-transformed (Cmax, AUC0-t, AUC0-∞) parameters were subjected to 90% confidence interval (CI) consistent with two one-sided t-tests. ANOVA of tmax, Cmax, AUC0-t, AUC0-∞ were subjected to a 4-way ANOVA accounting for sequence, subjects, period and treatment. Statistical significance was evaluated at 95% confidence level (p > 0.05). A certified validated WinNonlin version 3.0 program (Pharsight Corp., USA) was used for statistical evaluations of the pharmacokinetic parameters.

RESULTS AND DISCUSSIONS

HPLC analysis

The linearity of the calibration curve was determined by a weighted regression analysis. The coefficient of correlation for S-Etodolac varied from 0.9915-0.9986 (Table 4, 5). The response was found to be linear between concentrations of 1μg/ml to 8μg/ml. Inter-day and intra-day precision were determined from the low, medium and high QC samples. Precision is expressed as percent variation (% CV), while accuracy is measured as the percent nominal (% nominal). Intra-day precision results (% CV) for S-Etodolac ranged from 7.20 to 7.50. Intra-day accuracy (% nominal) results obtained at the low, medium and high QCs were 98.66, 99.73 and 98.37, respectively. The inter-day precision results (%CV) for S-Etodolac ranged from 5.59 to 7.46 and % nominal was 100.56, 102.81 and 100.12 respectively. The recovery of S-Etodolac was measured by comparison of the areas of etodolac after injection of the extracted sample with those obtained after injection of the standard solution containing equivalent concentrations of the drug. The percent extraction yield was obtained at low QC and high QC was 73.40 to 74.02 respectively.

Pharmacokinetic characteristics

The mean plasma S-Etodolac concentration time data from reference and test formulations are shown in Figure 1, Figure 2 and Figure 3. The semilog plot of the plasma concentration over time is shown in Figure 4. Cmax, AUC0-t, AUC0-∞ and Cmax/AUC0-∞ values were comparable in both formulations. The T/R ratio of geometric mean of Cmax, AUC0-t and AUC0-∞ for S-etodolac in both formulations was 106.94%, 103.93% and 105.64% respectively. (Table 1). These values were within the acceptance limit of 80 – 120%.

The ratio of AUC0-t and AUC0-∞ was higher than 80% in the most of the subjects after ingestion of test and reference formulation. In the case of reference formulation the ratio of AUC0-t and AUC0-∞ was below 80% for six subjects and In the case of test formulation it was below 80% for five subjects.

Clinical observations

No adverse effects were reported or observed. The post study clinical and laboratory evaluations showed no significant variations that could be attributed to treatment. Both formulations were thus well tolerated.

CONCLUSION

Test formulation containing S-Etodolac 300 mg (manufactured by Emcure Pharmaceuticals Ltd., Pune, India) was bioequivalent to S-Etodolac from reference formulation (Etodolac 600 mg, manufactured by Teva Pharmaceuticals, USA). Both formulations were well tolerated. The test formulation can be considered a pharmaceutically and therapeutically equivalent alternative to S-Etodolac from Etodolac.

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