Comparative bioavailability and pharmacokinetics of two oral formulations of flurbiprofen: A single-dose, randomized, open-label, two-period, crossover study in Pakistani subjects

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Abstract: Comparative bioavailability studies are conducted to establish the bioequivalence of generic formulation with that of branded reference formulation, providing confidence to clinicians to use these products interchangeably. This study was carried out to compare a locally manufactured formulation of flurbiprofen with that of a branded product. Twenty two healthy male adults received a single dose of flurbiprofen (100mg) either generic or branded product according to randomization scheme on each of 2 periods. Blood samples were collected and plasma flurbiprofen concentration was determined by a validated HPLC method. Pharmacokinetic parameters like $AUC_{(0\text{-}t)}$, $AUC_{(0\text{–}\infty)}$, C_{max}, T_{max}, t½, Vd and clearance were determined. The 90% CI for the ratio of geometric means of test to reference product's pharmacokinetic variables was calculated. Pharmacokinetic parameters for two formulations were comparable. Ratio of means of $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}\infty)}$ and C_{max} for test to reference products and 90% CI for these ratios were within the acceptable range. The p-values calculated by TOST were much less than the specified value (p-0.05). ANOVA gave p-values which were more than the specified value (p-0.05) for sequence, subject, period and formulation. Test formulation of flurbiprofen (tablet Flurso) was found to meet the criteria for bioequivalence to branded product (tablet Ansaid) based on pharmacokinetic parameters.

Keywords: Flurbiprofen, bioavailability, pharmacokinetics, bioequivalence, HPLC.

INTRODUCTION

Worldwide concern over the healthcare cost has lead to the rise in production of cheaper generic drug products. But at the same time, health professionals and drug regulatory authorities want these products to be as effective therapeutically as innovator drugs keeping in mind the benefit to the patients. For this reason, bioavailability and bioequivalence studies are carried out to establish the therapeutic equivalence of the generic product with that of branded innovator products (CPMP, 2000; FDA, 2003; Health Canada, 2004; SFDA, 2005; CDSCO, 2005; Dalen *et al.,* 2006; Benet and Larregieu 2010; Skelly, 2010; Mastan *et al.,* 2011).

Flurbiprofen is a non-steroidal anti-inflammatory drug belonging to phenylalkanoic acid derivative group and available as racemic mixture of R- and S-enantiomers. It possesses anti-inflammatory, analgesic as well as antipyretic activity due to its cyclo-oxygenase inhibiting action leading to prostaglandins biosynthesis inhibition (Kantor, 1986; Soraci *et al.,* 2005; Ozbay *et al.,* 2009; Alexander *et al.,* 2011). Flurbiprofen is effective in subsiding pain and inflammation in many conditions like osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, sunburn and also used post-operatively (Cherie-Lingniere *et al.,* 1983; Famaey and Ginsberg, 1983; Good, 1986; Charoo *et al.,* 2005; Richy *et al.,* 2007; Liu *et al.,* 2009; Sultan *et al.,* 2009; Bhaskar *et al.,* 2009). The pharmacokinetics of conventional dosage form of flurbiprofen have been studied in some populations including Pakistan (Kaiser *et al.,* 1986; Szpunar *et al.,* 1987; Caille *et al.,* 1989; Jamali *et al.,* 1991; Kean *et al.,* 1992; Greenblatt *et al.,* 2006; Qayyum *et al.,* 2011). Orally administered flurbiprofen is rapidly and almost completely absorbed making more than 95% of drug bioavailable (Szpunar *et al.,* 1987; Davies, 1995). It has extensive plasma protein binding of more than 99% which is responsible for its small volume of distribution (Kaiser *et al.,* 1986; Kean *et al.,* 1992; Hutzler *et al.,* 2001; Zgheib *et al.,* 2006). It has an elimination half life of about 7-8 hours measured with sensitive assay methods, although it was estimated to be between 3-4 hours by previous methods (Szpunar *et al.,* 1987; Adams *et al.,* 1987; Qayyum *et al.,* 2011). In humans flurbiprofen is eliminated primarily via oxidation and conjugation (Risdall *et al.,* 1978; Szpunar *et al.,* 1987; Jamali *et al.,* 1988). It is metabolized by cytochrome P450 2C9 and can be used as *in vivo* probe for phenotyping of CYP2C9 in humans (Kumar *et al.,* 2007; Petsalo *et al.,* 2008; Deglon *et al.,* 2011). Its metabolites are mainly excreted in urine (Szpunar *et al.,* 1987; Davies, 1995).

A new generic oral product of flurbiprofen (tablet Flurso 100 mg) was prepared locally to provide cheaper and effective product of flurbiprofen for patients. This

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bioequivalence study was conducted to compare the bioavailability and pharmacokinetic profile of this locally manufactured oral preparation with that of branded innovator product of flurbiprofen (tablet Ansaid 100/mg).

MATERIALS AND METHODS

Drug products and in vitro characterization

The generic or test product was tablet Flurso containing 100 mg of flurbiprofen manufactured by Amson Vaccines & Pharma Pvt Ltd, Pakistan. It was compared with the reference innovator brand of tablet Ansaid containing 100 mg of flurbiprofen (manufactured by Upjohn Parke, Davis & Co., Ltd. for Pharmacia Pakistan Pvt., Ltd). Weight variation, content uniformity and assay of active ingredients were conducted according to United States Pharmacopeia (USP) quality specifications (USP, 2010).

Study subjects

Twenty two healthy adult male subjects were recruited for the study according to the guidelines provided by the United States Food and Drug Administration (FDA, 2001, 2003). The age of the subjects was to be between 18-40 years and weight within 20% of normal body weight according to the Metropolitan Life Assurance tables. Each subject was evaluated to be healthy after a detailed medical history, physical examination and baseline laboratory tests. Subjects with history of smoking, drug abuse, any drug hypersensitivity and illness were excluded. All subjects were advised to avoid taking any over-the-counter medication for two weeks preceding the study. Subjects were informed of the nature, significance and consequence of the study and the investigational procedures. They gave informed consent by dated signature on the consent proforma.

Study protocol

The study was conducted in accordance with the current Good Clinical Practices (FDA, 1996) and the Declaration of Helsinki (WMA, 2008). The study protocol was approved by Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. It was a randomized, single dose, two-treatment, two-period crossover study. The subjects reported to the study area early in the morning after an overnight fast of 10 hours on each study period day. Each subject received a single dose of one tablet of flurbiprofen (100/mg) either test or reference product according to the randomization scheme on each of two study periods. There was a wash out period of 7 days in between two periods. Both test and reference drugs were of same strength (100/mg). Drug was swallowed orally with 240 ml of drinking water. Liquids were permitted after one hour of dosing. Standardized meal was served after 4 hours of drug administration. The standardization of study environment and diet was observed on both study periods.

Blood sampling

A 5ml blood sample was drawn immediately prior to and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hours after dosing on each of the two study period days. Each sample was transferred to heparinized tubes and was immediately centrifuged. Plasma was collected and frozen at -20°C until analysis.

Analytical procedure

Flurbiprofen extraction from plasma was accomplished by a validated HPLC method described by Qayyum *et al*. A brief account of method is given here. To 100/µl of plasma sample, 100/µl acetonitrile, 200/µl IS (naproxen 100 µg/ml) and 40/µl of half-strength phosphoric acid (50:50 phosphoric acid:water) added. After vortexing, samples were centrifuged at 14,000/g for 10 minutes and 150/µl were placed into autosampler vials. The autosampler was set to inject 20 µl onto the HPLC.

The High Performance Liquid Chromatography (HPLC) system by PerkinElmer with autosampler and fluorescence detector was used. Chromatographic separation was done on Chromolith® Performance RP-18 encapped column (4.6x100/mm, 5/µm particle size) at room temperature. The fluorescence detector was set at an excitation wavelength of 260 nm and an emission wavelength of 320 nm. The mobile phase consisted of acetonitrile (90%) -20/mM K_2HPO_4 (Dipotassium hydrogen phosphate) at pH 3.0 (40:60, v/v). The mobile phase was pumped at a flow rate of 2.5 ml/min. The retention time for flurbiprofen was 6.5 minutes and for naproxen it was 3 minutes.

The method was validated according to ICH Guidelines on validation of analytical procedures (ICH, 2005). Calibration curve was linear in the concentration range of 0.25-25 µg/ml, with correlation coefficient of 0.9992. The lower limit of quantitation was 0.25 µg/ml. Intra- and inter-day precision was determined by replicate analysis of standard samples in plasma containing 0.5, 10 and 25 µg/ml of flurbiprofen. Intra-day variability (co-efficient of variation) ranged between 3.23-9.49% and inter-day between 1.44-3.38%.

Pharmacokinetic analysis

Data of drug plasma concentration versus time for both formulations was used to calculate pharmacokinetic parameters by computer program, APO, MWPHARM version 3.60 (MEDIWARE Product, Holland). A two-compartmental open model was assumed to derive pharmacokinetic parameters. Area under the curve (AUC) from time 0 to t (AUC_{0-t}) was calculated by trapezoidal rule, where t is the last measured time point. AUC extrapolated to t-∞ was calculated using the relation Cm/β, where Cm is the last concentration measured for flurbiprofen and β is the slope of the least squares linear regression of the log concentration-time curve. The

maximum plasma concentration (C_{max}) and the time to reach it (T_{max}) were obtained directly by inspecting each individual plasma level-time curves.

STATISTICAL ANALYSIS

The pharmacokinetic parameters $AUC_{(0\text{-}t)}$, $AUC_{(0-\infty)}$ and C_{max} were analyzed statistically to determine if the two formulations were bioequivalent or not. The geometric mean ratios of test to reference products for AUC and C_{max} were calculated. The 90% confidence interval (CI) for the ratio of the geometric means of test to reference product's pharmacokinetic variables was calculated to determine whether values were within the accepted range of 80-125% for the log transformed data and within 80-120% for the original data. For this computer program Equiv Test 2.0 was used. The two one-sided t-tests (TOST) were carried out at 5% level of significance (α=0.05) to test the null hypothesis. ANOVA (analysis of variance) was performed on AUC and C_{max} using Minitab Release 13.1. ANOVA results revealed the effect of the sources of variation like sequence, subjects in a sequence, period and formulation on the bioequivalence data. The results of ANOVA were calculated at 5% level of significance (FDA, 1992, 2001; Riffenburgh *et al.,* 2006; Hauschke *et al.,* 2007).

RESULTS

The *in vitro* characteristics are summarized in table 1. Twenty two healthy adult male subjects participated in the study. The age of the subjects ranged from 20-38 years (mean 29.4±4.55) and weight from 50-94 kg (mean 70.09±2.44). None of the subjects reported any adverse effect of either formulation in both study periods. The mean plasma concentration-time profile for both formulations is plotted on linear and logarithmic scale as shown in fig 1 and 2, respectively. Mean pharmacokinetic parameters of flurbiprofen derived from the generic and branded products are summarized in table 2. The results of bioequivalence analysis in the form of geometric mean ratios of test to reference products for raw and log-transformed values of AUC and Cmax and 90% CI for these ratios is given in table 3. TOST with the null hypothesis of non-bioequivalence carried out at 5% level of significance gave the results shown in table 4. ANOVA results are summarized in table 5 taking into account the effect of sources of variation like sequence, subjects, period and formulation on the bioequivalence parameters.

DISCUSSION

All twenty two subjects showed detectable concentration of flurbiprofen at first sampling time of 15 min after drug administration for both the formulations. Flurbiprofen is a rapidly absorbed drug after oral administration as reported in other studies as well (Szpunar *et al.,* 1987; Davies, 1995). The flurbiprofen plasma-concentration time profiles of the generic and branded formulations were comparable. The pharmacokinetic parameters in these healthy Pakistani subjects were in agreement with the previously reported values (Kaiser *et al.,* 1986; Szpunar *et al.,* 1987; Caille *et al.,* 1989; Jamali *et al.,* 1991; Kean *et al.,* 1992; Greenblatt *et al.,* 2006). There may be differences in genetic profile and environmental conditions of Pakistanis and other world populations. The absence of any significant difference in these parameters implies that there is no substantial influence of environmental factors on the pharmacokinetic profile of flurbiprofen.

Table 1: *In vitro* characteristics of Flurbiprofen (tablet Flurso)

Description	Result	Specifications
Physical Appearance	Blue colour triangular tablet	Blue colour triangular tablet
Average weight of contents	290.2mg	--
Assay	105.22%	90-110%
Dissolution	82.53%	NLT75%
Content uniformity	Complies the test	85-115%

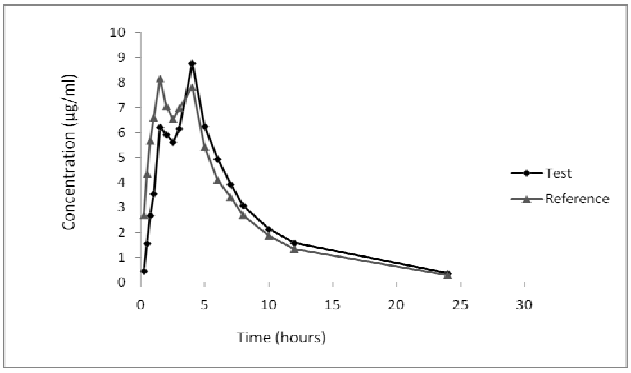

Fig. 1: Plasma concentration time curves of Flurbiprofen on linear scale.

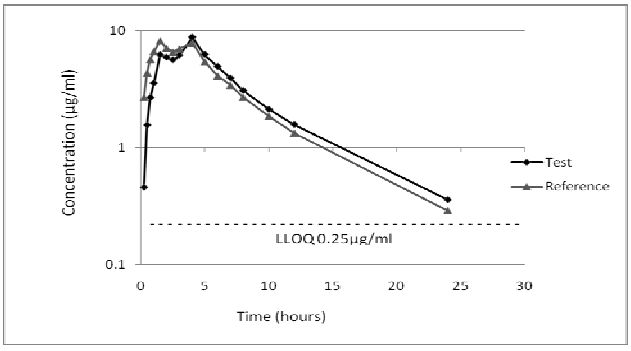

Fig. 2: Plasma concentration time curves of flurbiprofen on semilogarithmic scale

Bioequivalence of orally administered drugs is demonstrated *in vivo* by comparing the bioavailability of test product with that of reference standard product.

Bioavailability is determined by the rate and extent of absorption of a drug. The extent of absorption is evaluated by measuring AUC and the rate of absorption is assessed by observing C_{max}. So in bioequivalence studies AUC and C_{max} are analyzed statistically to determine if the test and reference products produce comparable values (FDA 1992, Nation and Sanson 1994; Welage *et al.,* 2001).

Table 2: Pharmacokinetic parameters of test (Flurso) and reference (Ansaid) drugs

Pharmacokinetic parameters	Test (Flurso) mean ± SD	Reference (Ansaid) mean±SD
$AUC_{(0-24)}$[a] (hr.mg/l)	62.01±21.68	61.31±21.31
$AUC_{(0-\infty)}$[b] (hr.mg/l)	59.89±28.76	62.15±38.19
Elimination half-life (hr)	5.36±3.43	6.85±7.89
Elimination rate constant (l/hr)	0.55±1.28	0.92±1.56
Time to peak concentration (Tmax) (hr)	2.91±1.10	2.43±1.28
Peak concentration (Cmax) (mg/l)	10.75 ± 2.55	12.04±3.21

[a]$AUC_{(0-24)}$ = area under the curve from time 0 to 24 hours; [b]$AUC_{(0-\infty)}$ = area under the curve from time 0 to ∞.

In our study the ratio of the geometric means of raw and log-transformed values of $AUC_{(0-24)}$, $AUC_{(0-\infty)}$ and Cmax for tablet Flurso (test/generic) to tablet Ansaid (reference/branded) were within the acceptable range (80-120%) for concluding bioequivalence for study products. The 90% CI for the ratio of means of raw and log-transformed values of $AUC_{(0-24)}$, $AUC_{(0-\infty)}$ and Cmax for tablet Flurso to tablet Ansaid were within the equivalence range of 80-120% and 80-125% for original scale and log-transformed data respectively. Therefore, equivalence with respect to both rate and extent of absorption can be concluded.

The two one-sided t-tests (TOST) procedure with the null hypothesis of non-bioequivalence at 5% significance level (α=0.05) was carried out. In case of original and log-transformed values of $AUC_{(0-24)}$, $AUC_{(0-\infty)}$, and Cmax, the *p*-values were calculated to be much less than the specified value of *p* (*p*-0.05), so both one-sided t-tests reject the null hypotheses of non-bioequivalence for lower and upper limits and the equivalence within the specified equivalence bound (80-125%) can be inferred.

The analysis of variance (ANOVA) was performed to account for any variability caused by sequence, subjects, drug or period on the equivalence results. The results of ANOVA were calculated at 5% level of significance (α=0.05). The ANOVA performed on original and log-transformed values of $AUC_{(0-24)}$, $AUC_{(0-\infty)}$ and C_{max} gave *p*-values which were more than the specified value of *p* (*p*-0.05) for sequence, subject, period and formulation. The results of ANOVA show that the sequence of the treatments, the subjects nested in a sequence, the drug products themselves and the periods of the study do not have any significant effect on the bioavailability of two products. So in this way they do not affect the bioequivalence results of our study.

CONCLUSION

Based on the accepted criteria for bioequivalence, the locally manufactured oral formulation of flurbiprofen, tablet Flurso (100/mg) is pharmacokinetically equivalent to the standard reference brand of flurbiprofen, tablet Ansaid (100/mg) in healthy Pakistani adults, so the bioequivalence of tablet Flurso with respect to tablet Ansaid can be concluded with confidence.

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Table 3: Bioequivalence parameters of Flurbiprofen

Parameters	Test mean ± SD	Reference mean ± SD	Ratio of Geometric means	90% confidence interval
$AUC_{(0-24)}$[a] (hr.mg/l)	62.01±21.68	61.31±21.31	101.20	94.12-108.68
ln[b] $AUC_{(0-24)}$	4.08±0.29	4.07±0.30	100.31	98.52-102.06
$AUC_{(0-\infty)}$[c] (hr.mg/l)	59.89±28.76	62.15±38.19	97.67	83.24-113.56
ln[b] $AUC_{(0-\infty)}$	4.01±0.4	4.03±0.4	99.41	96.65-102.26
C_{max}[d] (mg/l)	10.75±2.55	12.04±3.21	90.23	80.66-99.14
ln[b] C_{max}	2.35±0.21	2.45±0.27	96.02	91.82-100.09

[a]$AUC_{(0-24)}$ = area under the curve from time 0 to 24 hours; [b]ln = natural log; [c]$AUC_{(0-\infty)}$ = area under the curve from time 0 to ∞; [d]C_{max} = peak plasma concentration.

Table 4: Analysis of variance (ANOVA) of pharmacokinetic parameters of generic versus branded Flurbiprofen formulations

Pharmacokinetic Parameters/ Source of variation	df	Seq SS	Adj SS	Adj MS	F score	*p*-value
C_{max}[a]						
Sequence	1	19.752	19.752	19.752	2.53	0.122
Subject	10	94.000	94.000	9.400	1.20	0.329
Period	1	5.196	5.196	5.196	0.66	0.421
Formulation	1	18.331	18.331	18.331	2.34	0.136
Error	30	234.573	234.573	7.819		
Total	43	371.851				
$AUC_{(0-24)}$[b]						
Sequence	1	2190.4	2190.4	2190.4	5.99	0.020
Subject	10	6232.5	6232.5	623.3	1.70	0.126
Period	1	1.9	1.9	1.9	0.01	0.942
Formulation	1	5.4	5.4	5.4	0.01	0.904
Error	30	10974.5	10974.5	365.8		
Total	43	19404.8				
$AUC_{(0-\infty)}$[c]						
Sequence	1	2588	2588	2588	2.54	0.121
Subject	10	14356	14356	1436	1.41	0.224
Period	1	492	492	492	0.48	0.493
Formulation	1	56	56	56	0.05	0.816
Error	30	30567	30567	1019		
Total	43	48058				

[a]C_{max} = peak plasma concentration; [b]$AUC_{(0-24)}$ = area under the curve from time 0 to 24 hours;
[c]$AUC_{(0-\infty)}$ = area under the curve from time 0 to ∞.

Table 5: Two One-sided t-Tests (TOST) with Null Hypothesis of Non-bioaquivalence

Pharmacokinetic Parameters/ Null Hypothesis	t-value		p-value	
	Specified	Observed	Specified	Observed
$AUC_{(0-24)}$[a]				
NH-L[b]	1.7247	5.6108	0.0500	8.584E-06
NH-U[c]	-1.7247	-5.0644	0.0500	2.964E-05
$AUC_{(0-\infty)}$[d]				
NH-L[b]	1.7247	2.0593	0.0500	0.0263
NH-U[c]	- 1.7247	-2.8806	0.0500	0.0046
C_{max}[e]				
NH-L[b]	1.7247	1.7792	0.0500	0.04
NH-U[c]	-1.7247	-5.4806	0.0500	1.150E-05

[a]$AUC_{(0-24)}$ = area under the curve from time 0 to 24 hours; [b]NH-L=null hypothesis lower; [c]NH-U=null hypothesis upper;
[d]$AUC_{(0-\infty)}$ = area under the curve from time 0 to ∞; [e]C_{max} = peak plasma concentration.

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