Bioequivalence study of montelukast tablets in healthy Pakistani volunteers

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Abstract: Montelukast is a leukotrien receptor antagonist used for asthma treatment. Objective of this study was to evaluate the bioequivalence of two montelukast 10mg tablets, Innovator drug (Singulair) as reference and other locally manufactured drug (Montiget) in 12 healthy volunteers. It was randomized, single dose, two-period crossover study with 1 week washout period. Blood samples (4-5 ml) were collected before and after drug administration and plasma was separated for analysis. Concentrations of montelukast at different time intervals were determined by validated UV-HPLC method at 345nm wavelength. Bioequivalence was assessed by using non compartmental approach and also calculated the 90% confidence interval of the least-squared pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$). On average, C_{max}, AUC_{0-t}, AUC_{0-inf}, was 2.35µg/ml, 1.28µg.h./ml, 1.67µg.h./ml, for innovator drug and 2.53µg/ml, 1.53µg.h./ml, 1.96µg.h./ml, for test drug, respectively. Confidence interval (90%) for C_{max}, AUC_{0-t} and AUC_{0-inf} was 89-97%, 85–91% and 81-98% respectively. No statistical difference was found between the C_{max} and AUC values of test and reference drugs. The confidence intervals for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ are fully laid within the acceptable range of FDA (80-125%), thus two formulations are considered to be bioequivalent.

Keywords: Bioequivalence, pharmacokinetics, montelukast, Pakistani.

INTRODUCTION

Montelukast Sodium is competitively antagonize $cysLT_1$ receptor mediated broncho-constriction, increased vascular permeability and recruitment of eosinophils (Tripathy 2007). It blocks part of the inflammatory process associated with an asthma attack and thus helps to reduce swelling or constriction of airways. It is also used for the treatment of seasonal allergies. Leukotriene receptor inhibitors are used for asthma management and are compounds of a new pharmacological group and their discovery had made a major impact on strategies of asthma treatment (Cylly *et al*., 2003). Montelukast is also used as an additive therapy with inhaled corticosteroids for long term treatment of asthma and also produces good effects (Laviolette *et al*., 1999).

Montelukast has rapid absorption after oral administration. After administration of the 10 mg tablet peak plasma level is achieved in 2 to 4 hours (Schoors *et al.*, 1995; Cheng *et al*., 1996; Merck 1998). In 10 mg tablet bioavailability is about 64%, despite the consequences whether it is taken with food. Plasma protein binding of montelukast is 100% (Cheng *et al*., 1996; Merck 1998; Knorr *et al.,* 1998). Montelukast extensively metabolized by the cytochrome P450 enzyme system specifically CYP2C9and CYP3A4 in the liver. It is eliminated from body into bile (Cheng *et al*., 1996; Balani *et al.,* 1997; Chiba *et al*., 1997). The average half life of the montelukast in plasma is 2.7 to 5.5 h. Different gender difference studies were done but it was concluded that it did not affect the pharmacokinetics of montelukast (Cheng *et al*., 1996).

Bioequivalence studies helps to assess the therapeutic comparison of tested drugs (pharmaceutical alternatives or pharmaceutical equivalents). The significance of bioequivalence studies is increasing day by day due to the large availability of generic brands in market and their consumptions (Vetchý *et al*., 2007). Metabolic profiles can changes the pharmacokinetics of the drugs, so bioequivalence studies also help in study and comparison of metabolism of drug in different population (Srinivas *et al.*, 2009). Although several pharmacokinetic studies of montelukast have been published, only few studies are done on bioequivalence of montelukast (Sripalakit *et al*., 2010; Knorr *et al.*, 2010). Objective of present study was to compare a multinational company brand with locally manufacture brand of montelukast. The present study was designed to determine their pharmacokinetic parameters and compare them statistically to evaluate bioequivalence.

MATERIALS AND METHODS

Montelukast standard was gifted by Getz Pharma (Pvt.) Ltd., Karachi. All other chemicals of HPLC grade were purchased from Merck, Germany. Double distilled water was prepared in Bioequivalence Study Center, University of Veterinary and Animal Sciences, Lahore.

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Study subjects

Twenty four healthy Pakistani male volunteers participated in this study. Volunteers were selected according to inclusion criteria of the study. Age limit of the volunteers range between 19-30 years and body weight was more than 50 Kg. Medical history, physical examination and routine blood test showed that volunteers were good in health. Volunteers were non smoker and having no history of alcoholism. Volunteers were not allowed to take any medicine 10 days before and during the study phases to avoid the risk of drug interactions and the effects of induced or inhibited hepatic metabolizing enzyme. The study was conducted according to good clinical practices and after the approval by Ethical Committee of Bioequivalence Study Center, UVAS, Lahore. Participants who were fulfilling the inclusion criteria and provided voluntarily written consent on Informed Consent Form (ICF) and project information sheet were selected. Demographic data of volunteers is given in fig 1.

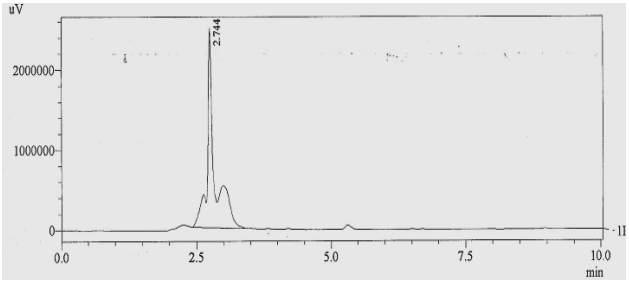

Fig. 1: Chromatogram of human blank plasma.

Study products

The study was conducted by using a test product (Montiget, Getz Pharma (Pvt.) Limited, Karachi; Batch # 054F13; Exp. Date: 12-13) which was a locally manufactured and a reference product (Singulair, Merck and Dhome, UK; Batch # 304848; Exp. Date: 09-13) which was the research brand of multinational company. 10mg oral dose of montelukast was given to each volunteer in the presence of the physician and clinical investigator. Volunteers were advised to inform immediately in case of any kind of side effect.

Study design

It was a single dose, two periods, two treatment, randomized crossover with washout period of one week**.** After overnight fasting, volunteers were divided to two groups, one group was given montelukast test (mont-test) drug and other one was given montelukast reference (mont-ref) drug (10 mg) by oral route. Blood samples (5ml) were collected at pre-dose '0' and at "0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10, 12 and 24 hours" after drug administration. Plasma samples were immediately separated after centrifugation and were stored at -80°C until analysis. After one week wash out period the same study was repeated with alternate treatment. High performance liquid chromatography (LC-20A, Shimadzu, Japan) was used for estimation of montelukast using a reverse phase column (LiChrospher 5 µm RP-18 column (250 x 4.6 mm), Merck, Germany).

Before analysis of montelukast, validation of the analytical method was done involving specificity, selectivity, accuracy and precision, lower limit of quantification (LLOQ), lower limit of detection, extraction recovery, linearity and stability.

Sample preparation

Plasma samples were extracted by taking 500 µl plasma and 500 µl acetonitrile in eppendorf tube, then centrifuged at 10,000 rpm for ten minutes. Supernatant was separated, filtered through 0.2µm filter paper and transferred to HPLC vials for chromatographic analysis.

Chromatographic conditions

Acetonitrile and 0.05 M potassium dihydrogen phosphate (80:20 v/v) was used as mobile phase with flow rate of 1 ml/min. The pH of buffer was maintained at 3.5 ± 0.1 with o-phosphoric acid. The temperature of the oven was maintained at 30^{o}C during the run process. Montelukast was monitored at 345nm and injection volume was set 70µl (Ibrahim, 2004).

Pharmacokinetic analysis

The plasma concentration vs time curve for each individual was used for data evaluation. Drug kinetic computer program named EquivTest PK was used for the evaluation of pharmacokinetic parameters using non compartment model. The kinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were taken for the bioequivalence evaluation between Mont-tst and Mont-ref products using 90% confidence interval. Analysis of variance (ANOVA) was used to check sequence effect on the test and reference drug product.

STATISTICAL ANALYSIS

To evaluate the significance of difference between two treatments and paired, t-test was used. The 90% confidence interval (CI) was used to evaluate the bioequivalence criteria. Drugs were considered bioequivalent when 90% confidence intervals were within the acceptable range of 80–125% for log-transformed pharmacokinetic parameters.

RESULTS

Selectivity

The selectivity of the method was examined by spiking the drug in blank plasma and ascertaining the retention time. The retention time of montelukast in spiked plasma was 6.6 ± 0.1 minute (fig. 2).

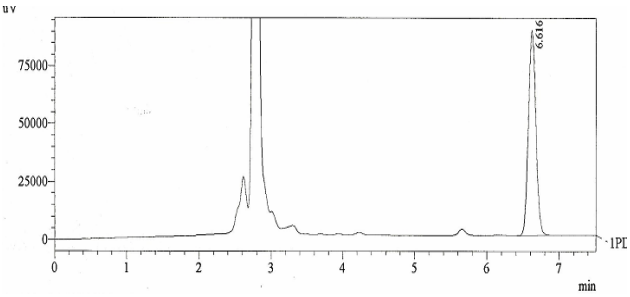

Fig. 2: Chromatogram of montelukast (200ng/ml) spiked in human plasma

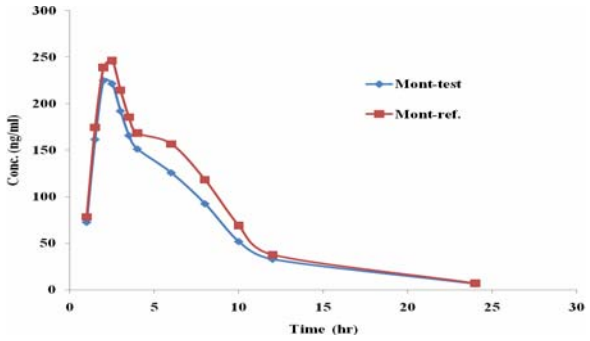

Fig. 3: Comparison of average plasma concentration of montelukast for test and reference drugs.

Linearity

Calibration of standard curve was done to obtain linearity of montelukast concentrations. Standard samples were prepared at concentration range 30-1000 ng/mL in drug free plasma. The standard curve showed a good linearity over the concentrations range which was observed: $y = 410.7x + 58819$, $r^2 = 0.997$.

Recovery

The recovery was measured for quality controls samples (low, medium and high) using six replicates. The average recovery of the assay was more than 85 %.

Precision and Accuracy

Interday and intraday precision of montelukast was also analyzed on the basis of quality control samples (n=6). The mean intra-day accuracy of low, medium and high quality control concentrations was 75.56, 113.49 and 114.28% while inter-day accuracy was 107.38, 110.78 and 110.38%. The %RSD of intra-day and inter-day precision of deferiprone were 4.43, 1.53, 0.19% and 1.50, 0.62, 0.14%.

Stability

Significant degradation of montelukast under the studied concentrations was observed in plasma. Stability of montelukast sample was also observed on light and heat exposure. It was concluded that montelukast was proved to be very sensitive compound (for both light and heat) and should be handled carefully.

Pharmacokinetics

Pharmacokinetic parameters of both test and reference products were not differ significantly shown in table 1. The 90% confidence interval was found to be 89 - 97% for C_{max}, 85 – 95% for AUC_{0-t} and 81-98% for $AUC_{0-inf.}$ ANOVA results showed that there was no significance variation among period, dug and sequence effect as shown in table 2. The mean ± SD plasma concentrations of montelukast for both test and reference drug obtained following oral administration to two groups of 12 volunteer are plotted in fig. 3.

Table 1: Mean pharmacokinetic parameters of both formulation (test and reference) of montelukast by non compartment model

Parameters	Mont-ref		Mont-test	
	Mean	S.D	Mean	S.D
Area Under the Curve $_{(0 \text{ to } t)}$ [h.mg/l]	1.53	0.59	1.64	0.80
AUC trapezoidal rule $_{(0 \text{ to inf})}$ [h.mg/l]	1.96	0.65	1.26	0.43
AUMC $_{(0 \text{ to } t)}$	8.90	6.56	7.58	3.88
Time to peak T_{max} [h]	1.58	0.19	1.63	0.23
Peak conc. C_{max} [mg/l]	0.253	0.04	0.235	0.47
Half-life [h]	4.9	2.62	4.63	3.45

Table 2: Analysis of variance (ANOVA) for AUC_{0-t}, C_{max} and $AUC_{0-\infty}$

Parameters	Inter-subjects		Intra-subject				F table
	Sequence		Period		Drug		
	F_{Value}	P_{Value}	F_{Value}	P_{Value}	F_{Value}	P_{Value}	
C_{max} (µg/ml)	3.72	0.08	0.26	0.62	9.59	0.01	4.96
AUC_{0-t} (µgh/ml)	1.45	0.26	0.05	4.96	14.02	0.00	
$AUC_{0-\infty}$ (µgh/ml)	1.35	0.27	1.13	0.31	3.92	0.07	

DISCUSSION

Due to influence of biochemical interior milieu of organism on the disposition and fate of drug, there has been increasing application of pharmacokinetic studies to describe the kinetics in specific environment and individualize the dose. In most cases the genetic makeup of indigenous population and environmental conditions are different from their foreign counterpart and this affects the biodisposition of drugs. So, evaluation of kinetic parameters is necessary to avoid the severe side effects.

The percentage recovery of quality control points of montelukast was found to be within the acceptable range. In case of precision the % CV (coefficient of variance) of inert and intraday analysis was also within the acceptable limit. It showed that these two parameters were properly validated. Standard curve obtained for montelukast concentration in plasma also showed good linearity with correlation coefficient (R^2) that was 0.997. Stability tests were also performed to properly validate the method that showed that montelukast is highly degradable in presence of heat and light.

All calculated pharmacokinetic parameter values for montelukast in the present study were in agreement with previously reported values (Cheng *et al*., 1996; Graff *et al*., 2003; Ochiai *et al*., 1998; Singulair, 2005; Sripalakit, 2008; Sripalakit, 2010). Pharmacokinetic parameters of montelukast in each individual following oral administration of test and reference products are shown in table 1. C_{max} and T_{max} are the parameters that show the absorption of drug, in this study C_{max} for montelukast with Mont-ref product was sighlty higher than that with Mont-tst product. However, T_{max} was almost the same in both products. AUC show that existence of drug in body and in this study AUC for both drugs was not different statistically. Different studies showed that elimination half life of montelukast was 2.5 to 5 hours and in this study the half life was 3.4h and 4.6 h respectively in test and reference drug that showed half life of montelukast in not different in Pakistani population. The values of C_{max}, AUC, and AUMC for montelukast showed slight difference between the 2 products. However, the pharmacokinetic profile obtained following oral administration of Mont-ref product was significantly similar from those of Mont-tst.

No statistical difference was found between the C_{max} and AUC values of test and reference drug obtained by ANOVA for two-way cross over study and 90% confidence interval. The confidence intervals for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ are fully laid within the acceptable range of FDA (80-125%). From the ANOVA test of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ it was found that no significant difference in drug, sequence, subject or period effects as shown in table 2.

CONCLUSION

It is concluded that the bioequivalence parameters (AUC and Cmax) obtained after oral administration of mont-tst and mont-ref were statistically same. The confidence intervals for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ are fully laid within the acceptable range of FDA (80-125%), thus two formulations are considered to be bioequivalent.

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