PHARMACOKINETIC COMPARISON OF MONTELUKAST SODIUM FORMULATIONS AFTER A SINGLE ORAL DOSE IN HEALTHY GUINEA PIGS

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ABSTRACT

Objective: Pharmacokinetic evaluation of montelukast sodium chronomodulated capsules (sustained-release solid dispersion of drug enclosed in pH-sensitive film-coated hard gelatin shell) and marketed tablets has been carried out in this study.

Methods: A single oral dose of prepared capsules and marketed conventional tablets was administered in healthy male Dunkin-Hartley albino guinea pigs. Blood samples were collected at different time intervals and plasma concentration of drug was determined by reversed-phase high-performance liquid chromatography. Different pharmacokinetic parameters were assessed from plasma drug concentration-time profile by one-compartment model, first-order kinetics.

Results: Pharmacokinetic parameters such as time to reach maximum concentration, elimination rate constant, elimination half-life, and mean residence time data indicates that drug release from chronomodulated capsules is significantly prolonged with initial release lag time of 3.5–4 h in comparison with marketed conventional tablets. However, maximum drug plasma concentration, area under the concentration-time curve, and apparent volume of distribution values show non-significant difference between capsules and marketed tablets.

Conclusion: The findings specified that capsules were providing time controlled delivery of drug at a desired rate for prolonged time, which may be helpful for the prevention of episodic attack of asthma in early morning hours.

Keywords: Montelukast sodium, Pharmacokinetics, High-performance liquid chromatography, Guinea pig plasma, Sustained-release solid dispersion, pH-sensitive coating.

INTRODUCTION

Pharmacokinetics is a study of drug disposition in the body and focuses on the changes in the blood drug concentration over time [1]. The pharmacokinetic characteristics can be quantitatively expressed by its parameters such as maximum drug plasma concentration, time to reach maximum concentration, area under the concentration-time curve (AUC), elimination rate constant (K_{el}), elimination half-life ($t_{1/2}$), mean residence time (MRT), clearance, and apparent volume of distribution (V_d). Compartment models are used in pharmacokinetics as a tool to compute and analyze the parameters that help to describe the experiment results [2].

Montelukast sodium (MS) is a leukotriene receptor antagonist and advised for once-daily oral dosing for the management of symptoms associated with asthma in children and adults [3,4]. Montelukast is an effective, well-tolerated alternative to inhaled corticosteroids treatment in patients with mild asthma who are uncontrolled or unsatisfied with low-dose inhaled corticosteroids therapy [5]. MS salt has a high-molecular-weight (608.169 g/mol), with solubility in water is approximately at a concentration of 10 mg/ml [6]. Montelukast works by blocking a chemical reaction that perpetuates inflammation in airways [7]. This is an acidic drug which is highly bound to plasma albumin and 64% bioavailable orally [8,9]. The main drawback of conventional montelukast formulation is that it undergoes hepatic first-pass metabolism and shows biological half-life of 2.5–5 h [10]. Chronomodulated capsules (sustained-release solid dispersion of drug enclosed in pH-sensitive film-coated hard gelatin shell) are designed to have initial 3.5–4 h release lag time with sustained release up to 10 h assuming that capsules to be consumed at bedtime. This approach was consistent with the demand of drug during early morning hour attack of asthma. Asthma is a chronic inflammatory disorder of the airways which has significant effect on patient's health and quality of life [11].

The present study brought out the pharmacokinetic characteristics of MS chronomodulated capsules administered to guinea pig.

MATERIALS AND METHODS

Materials

MS was received from Sanofi Aventis, Goa, India, as a gift sample. Metoclopramide hydrochloride was obtained from Vaikunth Chemicals Pvt., Ltd., Gujarat, India. All other chemicals and reagents were of analytical grade or high-performance liquid chromatography (HPLC) grade procured from E-Merk (India) Ltd., and Fluka chemicals.

Methods

Pharmacokinetic design and analysis

Institutional animal ethical committee (1044/PO/Re/S/07/CPCSEA, ITS/01/IAEC/2017) I.T.S College of Pharmacy, Ghaziabad, was approved the study protocol.

Chromatographic conditions

The analysis was carried out reversed-phase HPLC, equipped with intelligent ultraviolet (UV)-visible spectrophotometry detector (Jasco UV-2075, Japan), autosampler, Cosmosil5C18-MS-II (4.6 ID×250 MM) column, and pump (Jasco Intelligent Pump, 2080 Plus, Japan). Column temperature was set as 25–27°C, run time; 10 min, and wavelength; 287 nm. The mobile phase consisted of the mixture of acetonitrile

and sodium acetate buffer (20 mM, pH adjusted to 5.0) in the ratio of 80:20 v/v was delivered at a flow rate of 1.5 ml/min, isocratically. Ultrasonic bath was used to remove dissolved gases and entrapped air in mobile phase. Vortex mixer (Remi CM-101 Plus, India) and cooling centrifuge (Remi CM-12 Plus, India) were used for sample processing.

The bioanalytical method was adopted as described earlier by Ranjan *et al.*, 2013, and Shafaati *et al.*, 2010, after performing partial method validation [12,13].

Plasma sample preparation for calibration curve
Calibration curve of MS was prepared by spiking of 800 μl of the guinea pig plasma samples with 100 μl of previously prepared working solutions of concentration of 1, 2, 5, 10, 15, and 20 μg/mL, keeping internal standard (metoclopramide hydrochloride) solution concentration 8 μg/mL in each one. Accordingly, plasma samples contain the final concentration of MS 100, 200, 500, 1000, 1500, and 2000 ng/mL, respectively, with 800 ng/mL of metoclopramide hydrochloride as internal standard in each [14,15].

MS was extracted from plasma sample by protein precipitation technique using saturated NaCl solution [16]. 2.5 ml of saturated
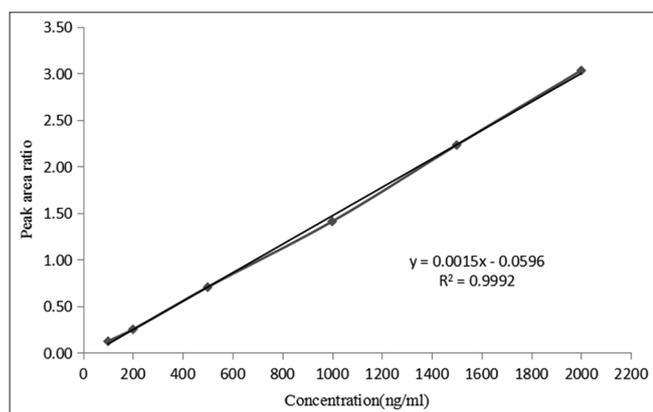
solution of NaCl was mixed with 1 ml of spiked plasma sample. The mixture was vortexed for 15 min followed by centrifugation at −4°C, 8000 rpm for 20 min. Finally, I injected 20 μl of supernatant into HPLC column [12,13].

Study design, drug administration, and blood sampling
The study was carried out in six male Dunkin-Hartley albino guinea pigs of average weight 562 g. The animals were housed with free access to standard food and tap water *ad libitum*. The experimental animals were housed in air-conditioned rooms at 21–23°C and 60–65% of relative humidity and provide a regular light/dark cycle. Animals were handled as per guide for the care and use of laboratory animals and left to acclimatize for 2 weeks before the start of the experiment [17]. The overnight fasted pigs were divided into two groups having three animals in each group. One group was fed with developed capsule and other with marketed conventional tablet as a single oral dose [18-20]. After washout period of 20 days, the pigs were crossed over and administered the alternate formulation. All the animals had free access of food and water after 4 h of drug dosing. After a single oral administration, blood was collected in ethylenediaminetetraacetic acid coated tube at different time intervals [21]. Blood was immediately centrifuged (Remi CM-12 Plus, India) at 8000 rpm for 20 min at −4°C to separate plasma, and plasma samples were stored at −20°C±2°C for further analysis [22].

Analysis of blood samples
Frozen plasma samples were first thawed at room temperature and then spiked with internal standard, for this 20 μl of solution containing 8 μg/mL of an internal standard were added to 230 μl of each plasma sample, followed by drug extraction by saturated solution of NaCl. Plasma concentration of MS was calculated from regression equation and pharmacokinetic parameters were determined by one-compartment model, first-order kinetics [23,24]. Pharmacokinetic parameters were calculated by the following method: AUC by linear-log trapezoidal method, $MRT_{0-t}=AUMC_{0-t}/AUC_{0-t}$, $MRT_{0-\infty}=AUMC_{0-\infty}/AUC_{0-\infty}$, and $Cl= dose/AUC_{0-t}$, K_{el} using equation $\ln(C0/Ct)=K_{el} t$, where, C_0 is initial concentration, C_t is concentration at time t, and $V_d=dose/k_{el}[AUC]_{0-\infty}$.

Data and statistical analysis
The concentration-time data were collected and entered into MS Excel 2010 worksheet. SPSS version 16.0 was used for statistical data analysis. The descriptive statistics (mean, standard deviation, and standard error

Fig. 1: Calibration curve of montelukast sodium in guinea pig plasma

y = 0.0015x - 0.0596
R² = 0.9992
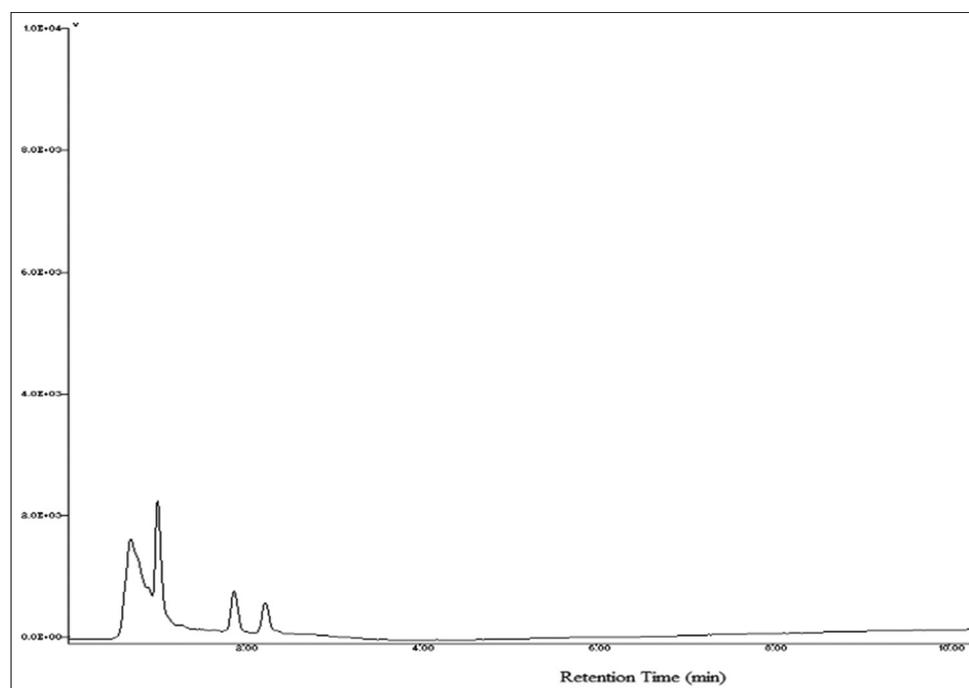
Fig. 2: Representative chromatogram of drug-free blank guinea pig plasma
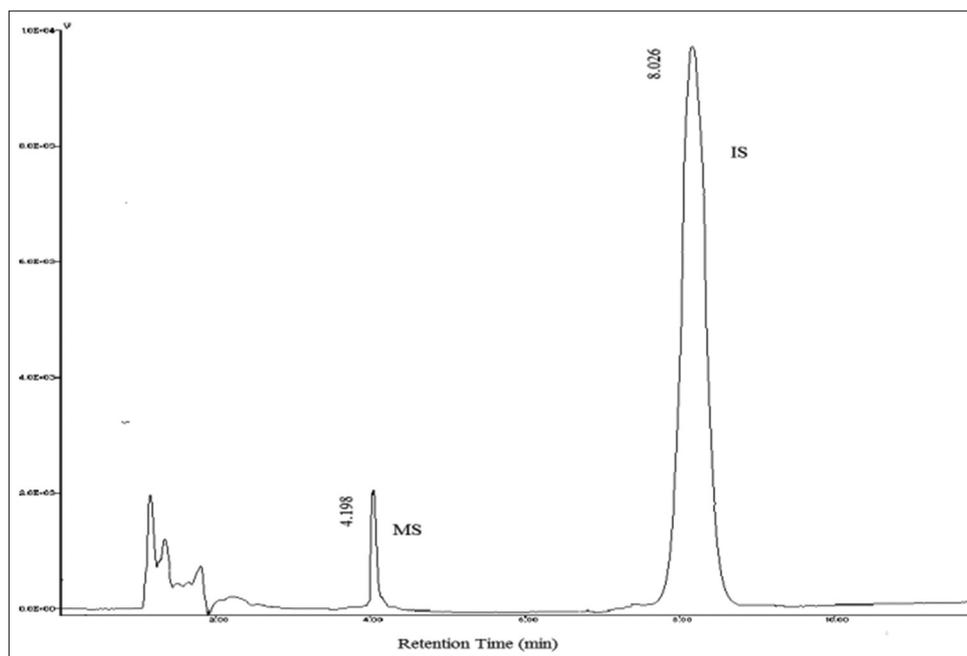
Fig. 3: Chromatogram of plasma spiked with internal standard 800 ng/ml and montelukast sodium 100 ng/ml
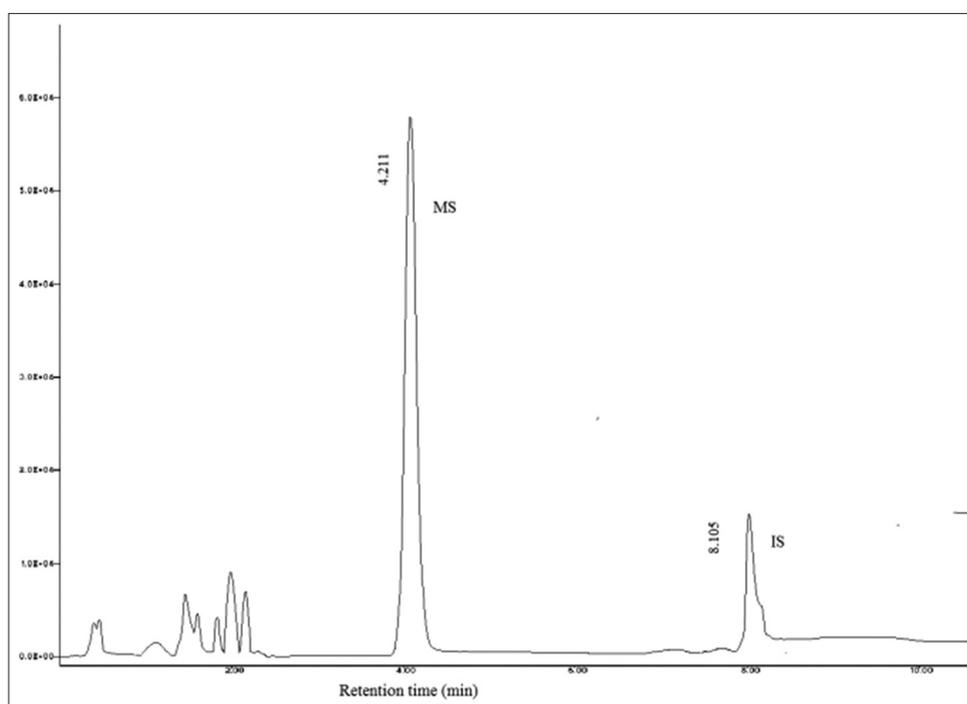
Fig. 4: Plasma spiked with internal standard 800ng/ml and montelukast sodium 1200 ng/ml

of mean) were calculated. The normality of data was tested by Shapiro–Wilk test and data were found normal; hence, to test the significance of mean differences of pharmacokinetic parameters between two independent groups, unpaired t-test was used. The level of significance and confidence interval was accepted as 5% and 95%, respectively [25,26].

RESULTS

The ratio of peak area of the MS to the peak area of the internal standard has given a linear correlation over a concentration range of 100–2000 ng/ml with correlation coefficient (r^2) of 0.999 (Fig. 1). The mean regression equation was y=0.001x-0.0590, where, y is peak area ratio and x is the concentration of MS in plasma.

Three drug substances were examined to achieve suitable internal standard for guinea pig plasma analysis, among them metoclopramide hydrochloride was found as most suitable for quantitation of MS level. Representative chromatogram of blank plasma in comparison to spiked samples of MS at concentration ranging from 100 ng/ml to 12000 ng/ml with internal standard at a constant concentration of 800 ng/ml is shown in Figs. 2-4. Chromatograms confirmed that MS and IS were well separated at retention times of 4.19–8.10 min, respectively, under the described chromatographic conditions.

The mean pharmacokinetic parameters computed from the individual pharmacokinetic analysis of the MS plasma concentration-time data of each animal are tabulated in Table 1.

Table 1: Pharmacokinetic parameters of MS in chronomodulated capsules and marketed conventional tablets in guinea pigs, all values are expressed as mean±SEM, n=6

Parameters	Results	
	Chronomodulated capsules	Conventional tablets
C_{max} (ng/ml)	515.29±2.16	525.23±4.06*
T_{max} (h)	6.00	2.00
$T_{1/2}$ (h)	7.52±1.94	4.30±0.29
AUC_{0-t} (ng h/ml)	4500.52±29.19	3161.96±52.17*
$AUC_{0-\infty}$ (ng h/ml)	5007.25±36.36	4015.67±43.75*
K_{el} (1/h)	0.09±0.012	0.16±0.014
V_d (L)	0.94±0.08	0.73±0.05
MRT_{0-t} (h)	10.86±1.73	6.67±0.91
$MRT_{0-\infty}$ (h)	11.93±2.18	7.59±1.31

*Non-significance difference was observed. Vd: Volume of distribution, SEM: Standard error mean, C_{max}: Maximum drug plasma concentration, T_{max}: Time to reach maximum concentration, $T_{1/2}$: Elimination half-life, MRT: Mean residence time, K_{el}: Elimination rate constant, AUC: Area under the concentration-time curve, MS: Montelukast sodium
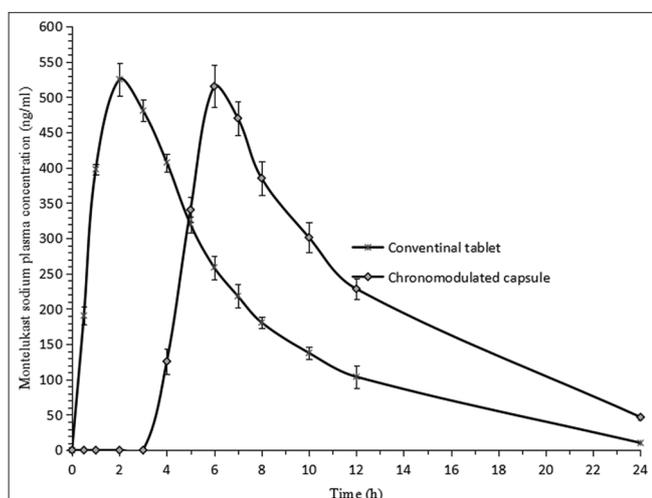
Fig. 5: Plasma concentration-time profile of montelukast sodium in chronomodulated capsules and conventional tablets

The pharmacokinetic profile of chronomodulated capsules was compared with that of marketed conventional tablets (Montair 10, by Cipla Ltd., India) at equivalent dose and shown in Fig. 5.

DISCUSSION

Conventional tablets reached to peak plasma concentration within 2 h compared to 6 h for the chronomodulated capsules after a single oral administration. Furthermore, chronomodulated capsules demonstrated significantly longer $t_{1/2}$ then the conventional tablets. Conventional tablets are immediate dosages form and release drug in gastric pH, but capsules release the drug in the higher pH 6.8 after dissolution of coating. Sustained-release pattern of capsules after lag release time of 3.5 h leads to more uniform drug absorption and slow elimination as compared to conventional tablets. MRT was significantly prolonged for capsules due to slow elimination rate. Capsules showed complete release of drug in 12 h in a controlled manner by diffusion mechanism. The mean C_{max}, T_{max}, $t_{1/2}$, AUC, MRT, V_d, and Ke values obtained in the present study (Table 1) are comparable to that described in other similar studies [26-29]. To determine bioequivalence of developed capsule with marketed conventional tablet in single-dose studies, C_{max} and extent of absorption (AUC) were considered. The absence of a significant difference in the C_{max} and AUC at equivalent dose under the same conditions and same route of administration establishes the bioequivalence between the two formulations in this study.

CONCLUSION

The *in vivo* study indicates that capsules release the drug in sustained manner after initial predetermined lag time and may be useful for the better management of asthma.We compared the bioavailability of prepared capsules with that of commercial tablets and results show that the two studied formulations are bioequivalent with respect to Cmax and AUC.

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AUTHORS' CONTRIBUTIONS

G T Kulkarni designed the study protocol and Neelam Singh collected the data, performed the analysis, and wrote the paper. Y Kumar contributed in interpretation of data and getting approval by the ethical committee.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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