Research Article

An *In-vivo* Pharmacokinetic Evaluation of Chrono Pharmaceutical Drug Delivery System of Carbamazepine for Epilepsy

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ABSTRACT

Chrono therapeutics refers to treatment method in which *in-vivo* drug availability is designed to match the circadian rhythms of disease to improve therapeutic outcomes and minimize adverse effects. The circadian patterns can influence the pharmacokinetics of certain drugs shown in clinical studies used in the treatment of diseases that follows circadian rhythms. The bioavailability of such drugs is influenced by the time of administration. The objective of the present study is pharmacokinetic evaluation of compression coated carbamazepine tablets and marketed conventional carbamazepine tablets. Carbamazepine is the first line drug of choice in the treatment of epilepsy. The oral bioavailability of carbamazepine is decreased upon repetitive doses due to hepatic auto induction. Hence compression coated carbamazepine tablets were prepared. The prepared dosage forms of compression coated tablets and marketed conventional tablets of carbamazepine were administered to 2 groups of white New Zealand rabbits (n=6) following cross over design pattern. Different pharmacokinetic parameters were assessed from plasma drug concentration time profile by using Kinetica software. The pharmacokinetic software such as Cmax, elimination rate constant, elimination half-life and mean residence time. The plasma drug levels of compression coated tablets of carbamazepine showed an initial lag phase of 3-4 hours before releasing the drug in comparison to marketed conventional carbamazepine tablets. The compression coated tablets showed maximum time for peak plasma concentration (Tmax) at the 7th hour in comparison to marketed conventional tablets at 3 hours. However, area under the curve, apparent volume of distribution show non-significant differences between Compression coated tablets and conventional marketed tablets of carbamazepine. These findings specified that compression coated tablets were providing timed pulsatile drug release at specific times to match the circadian rhythms which may be helpful for the prevention of episodic seizure attacks of epilepsy occurring during circadian timings of the day.

Keywords: Chrono therapeutics, *In-vivo*, Pharmacokinetic Parameters, Pulsatile, Compression coated tablets, lag-phase.

INTRODUCTION

Chrono therapy refers to the treatment method which has the ability to meet the therapeutic concentrations of the drug in accordance with a patients biological rhythmic changes occurring during daily, monthly, seasonal or yearly and thus minimizing the adverse effects[1]. Chrono therapeutic drug delivery system with a predetermined lag time are helpful for patients suffering from Epilepsy, Allergic rhinitis, Rheumatoid arthritis and related disorders, Asthma, Cancer and Cardiovascular disorders as the drug is released with a lag phase where the maximum concentration of drug (C max) will be available in the therapeutic concentrations when the symptoms of the disease are at its peak [2-4].

Epilepsy is a disorder of the brain caused by disturbances in the normal patterns of neuronal activity, imbalances of neurotransmitters symptomatised by uncontrolled recurrent seizures or without loss of consciousness involving apart or whole part of the brain[5]. Epileptic seizures were observed to exhibit circadian periodicity in patients from the past 2000 years[6]. Depending upon the origin of seizures in the brain epileptic seizures exhibit circadian rhythmicity [7-11].

Epileptic seizures were observed to show circadian rhythms during 11:00H to 17:00H and from 23:00H to 05:00H (12). Chrono pharmacotherapy for epilepsy has been suggested to ensure that the therapeutic concentrations coincides with the circadian patterns of epilepsy when the severity of the disease is at its peak. Compression coated tablets are dosage forms consisting of an inner immediate release core tablet embedded in an outer layer made of a hydrophilic or hydrophobic layer or a combination of both. The outer layer disintegrate slowly to release the drug after a pre-determined lag phase [13-14].

Compression coated tablets technique is comparatively simple, economical and prevents the need for laborious and prolonged granulation or coating process. The technique also improves the stability of the drug by shielding it from moisture[15]. Current drugs that are available in the market are conventional, sustained or controlled release tablets for epilepsy and these dosage forms needed to be taken repetitively depends especially during the late afternoon hours and early morning hours of the day. This necessitates the present study for the compression coated tablets and to study the differences in pharmacokinetic parameters of compression coated

carbamazepine tablets and conventional marketed carbamazepine tablets.

MATERIALS AND METHODS

Carbamazepine was a generous gift sample from Aurobindo pharmaceuticals private limited, Hyderabad. Methanol- HPLC grade, potassium dihydrogen phosphate –AR grade, Milli Q water-HPLC grade (SD fine chemicals ltd, India), sodium heparin, 0.22 µm nylon6,6 membrane filter, microtips (200µl-1000µl), RIA vials and microtips (200µl-1000µl).

Methods

Compression coated carbamazepine tablets (100mg) containing Eudragit L-100 and Eudragit S-100 prepared in the laboratory and conventional marketed carbamazepine tablets were chosen on the basis of drug content and lag time achieved, *in vitro* drug release studies and stability conditions were chosen as dosage forms for administration.

Animal studies

The pharmacokinetic study was performed on male New Zealand white rabbits (2.5-3.0 kg) used in the study which are chosen after animal ethical clearance certificate from CPSCEA committee.

Each group consisting of six rabbits (n=6) and were divided into two groups (A and B). The rabbits were kept in polycarbonate cages in a room maintained under constant environmental conditions food and water were given ad libitum. The selections of number of number of animals were done as per the recommendations of IAEC group. In the present study Group I were administered with compression coated carbamazepine tablets and Group II rabbits were administered with conventional Marketed Carbamazepine tablets [16-20].

Cross over design was employed in the study with a wash out period of three weeks in between the two experiments. Sterile internal stomach pumps were used for administering the dosage forms.

Blood sampling

The rabbits were fasted overnight for 12 hours prior to the study. The animals were fed 4 hours after the dosing of drug, after which the animals were given free access to water throughout the period of the study. Blood samples of 5ml were withdrawn from the marginal ear vein and collected into heparinized tubes at pre-determined intervals of 0min (pre-dose), 15, 30, 60, 120, 180, 240, 480, 600, 720, 1200 and 1440 minutes. The plasma was immediately separated by centrifugation at 4000 Rpm for a period of 5min and were frozen at -20^{0}c until the sample is analyzed.

Preparation of internal standard (IS) stock solution

About 10 mg of internal standard (Propyl paraben) was weighed accurately and transferred into a volumetric flask. It was then dissolved in methanol and the volume was made up with the same to produce a solution of 1mg/ml strength of carbamazepine. The above final concentration for carbamazepine was corrected for accounting its potency and the actual amount weighed. It was then stored in refrigerator.

Preparation of carbamazepine standard stock solution

10 mg of accurately weighed carbamazepine working standard was transferred into a 10 ml volumetric flask dissolved in methanol and the volume was made up to the mark with methanol to produce stock solution of 1mg/ml strength of carbamazepine. The above final concentration for carbamazepine was corrected for accounting its actual potency and the actual amount weighed. It was then stored in refrigerator.

Spiking of plasma for samples

In a 10 ml volumetric flask 0.7 ml of each of the described stock solution of carbamazepine was taken and was made up to volume with sodium heparin. The plasma was then pooled and mixed well.

Sample preparation

From the deep freezer all the samples of one or more periods and of one or more subjects were withdrawn and allowed them to thaw at room temperature. After thawing the samples were vortexed for ensuring complete mixing of contents. Samples of 1000µl were pippeted into respectively labelled radio immune assay vials. Now 50µl of internal standard (0.5 µg/ml) were added into respectively RIA vials and labelled.

To the labelled RIA vials 0.5ml of ethyl acetate as extraction solvent was added and capped. All the samples were placed in vibrimax for 10 minutes at 2500 rpm. The samples were centrifuged for 5 min at 10000 rpm in a refrigerator centrifuge. Organic layer of 0.4 ml was transferred into respective radio labelled vials. The samples containing organic layer were exposed to nitrogen evaporator at 40°C. The dried organic residue was reconstituted with 0.1 ml of mobile phase and vortexed. Reconstituted samples were transferred into respectively labelled vials. 20µl of the above samples were injected into the HPLC system using the chromatographic conditions described below in Table 1.

Table 1: Chromatographic conditions of HPLC

Apparatus Parameters	Specification
Apparatus	Shimadzu LC 2010
Column	C18* 125*4.0 mm
Mobile Phase	30Mml phosphate buffer (5% phosphoric acid) and acetonitrile (65:35)
Column Temperature	Ambient
Injection volume	5µl
Flow rate	1.0 ml/min
Run time	15.0 min

Chromatographic conditions

The column composed of C18 (4.6*150mm), mobile phase composed of 30 Mml phosphate buffer (5% phosphoric acid) and acetonitrile (70:30) 700 ml of phosphate buffer and 300 ml of acetonitrile 70:30% v/v mixture, the injection volume was about 20µl, the flow rate was about 1.0 ml/min and the run time was fixed for 15 min. The column oven temperature was about 40^{0}c and the sample cooler temperature was fixed at 10^{0}c.

Data analysis

Pharmacokinetic parameters like peak plasma concentration (Cmax), time to reach peak plasma concentration (T max), Area under the curve (Auc $_{0-12}$), (Auc$_{12-24}$) Area under the first moment curve (AUMC$_{0-24}$), Elimination half-life ($T_{1/2}$) and Mean residence time (MRT) were calculated using Kinetica software.

Calculation of the concentration.

The concentration of the unknown was calculated from the following regression analysis method of spiked plasma calibration standard with the reciprocal of the square of drug concentration as weighing factor.

(1/concentration *concentration).

Y= mx+b

x=concentration of the analyte

m= slope of the calibration curve.

Y=peak area ratio of analyte to internal standard (IS).

B= y axis intercept of the calibration curve.

Table 2: *In vivo* comparative pharmacokinetic parameters of Compression coated carbamazepine tablets and Conventional marketed formulation

Pharmacokinetic parameters	Carbamazepine formulation C_5	Marketed formulation MF
Auc 0-∞(µg*h/mL)	116.22±22.86	163.14± 39.19
Auc 0-t(µg*h/mL)	116.2199609±20.82	163.1420276±38.7
Elimination rate constant(1/h)	1.041031954±0.12	0.520523546±0.13
T1/2	0.665685618±0.23	1.331351876±0.25
volume of distribution (L/kg)	0.895741036±0.35	0.638121456±0.31
clearance(L/h)	0.860435679±0.45	1.225922364±0.43
Cmax µg/ml	31.48761013±10.63	21.95055446± 12.0
Tmax (h)	7±0.2	3±0.2
MRT(h)	8.22±0.24	5.30±0.36
AUMC µg*h/mL)	955.5625± 102.0621	865.551 ± 104.5116

RESULTS AND DISCUSSION

The initial goal for developing any dosage form is to deliver the drug in required concentrations for attaining optimum therapeutic concentrations. The ability of the compression coated carbamazepine tablets to deliver drug in a pre-determined lag phase was investigated in New Zealand white rabbits. The Compression coated carbamazepine tablets and conventional marketed carbamazepine tablets were taken for pharmacokinetic studies. Chromatogram of carbamazepine drug in Rabbit serum is shown figure 2 Mean plasma drug concentrations vs time as shown in figure 1 of all the groups of rabbits were studied for comparing various pharmacokinetic parameters. Maximum plasma drug concentration (Cmax) and the time to maximum peak plasma concentration (Tmax) were obtained directly from the plasma drug profile for each animal following administration of the above mentioned Compression coated carbamazepine tablets and conventional marketed carbamazepine tablets.

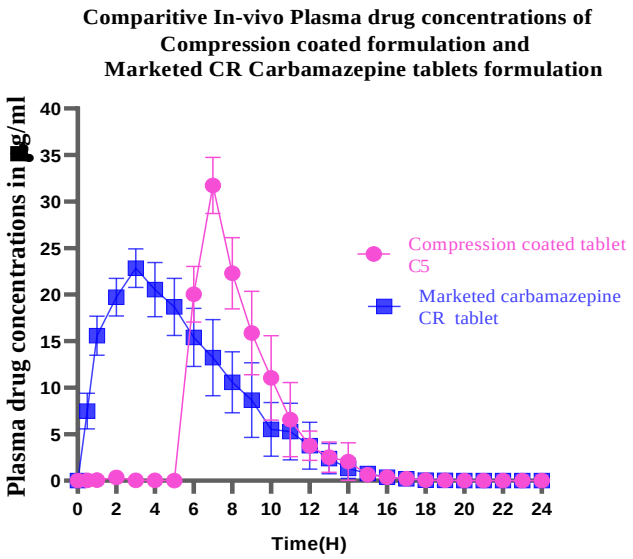

Figure 1: *In-vivo* plasma drug concentrations of Carbamazepine compression coated tablets and marketed CR carbamazepine tablets

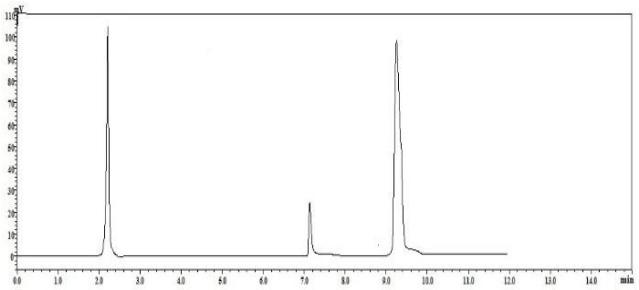

Figure 2: Chromatogram of carbamazepine standard in rabbit serum

The AUC O-∞ for animals administered with Compression coated tablets C5 [21] of carbamazepine were found to be 116.22±22.86 (µg*h/ml) and the animals given marketed conventional carbamazepine tablets were found to be 163.14± 39.19 (µg*h/ml).

MRT is defined as the mean time for the intact drug molecule to transit through the body and involved in a composite of all kinetic process that includes release and absorption of drug followed by drug disposition. The in-vivo performance of compression coated tablet can be used to evaluate in a comparative way with conventional marketed carbamazepine tablets. The MRT of compression coated carbamazepine tablets C5[21] was found to be 8.22±0.24 hrs. While that of conventional Marketed Carbamazepine tablets was found to be 5.30±0.36 hrs. The increase in the MRT was mainly due to change in drug release and elimination. The average Tmax values were found to be 7±0.2 hrs. for compression coated carbamazepine tablets and for conventional marketed carbamazepine tablets was 3±0.2 hrs. The marketed conventional carbamazepine tablets showed low Tmax values due to faster drug absorption in comparison to compression coated carbamazepine tablets. According to the summary of pharmacokinetic parameters given table 2 suggests that the compression coated carbamazepine tablets showed a lag phase of 3-4 hours before finally showing maximum drug concentration31.48±10.63 µg/ml at 7±0.2th hour which correlated with invitro drug release in comparison to conventional Marketed Carbamazepine tablets showed Cmax of 21.95055446± 12.0 µg/ml at 3±0.2th hour.

One way analysis of variance (ANOVA) using Dunnet multiple comparison test on computer program graph pad instat 3 was used. The differences were considered significant at p-values equal or less than 0.05 p≤0.05.

CONCLUSION

The developed RP-HPLC method was relevant and suitable for the detection of carbamazepine in plasma in concentrations of 0.5 µg/ml. In conclusion compression coated carbamazepine tablets release drug over a period of 4-12 hours consistent with the requirements of Chrono pharmaceutical drug delivery was attained to suit the circadian patterns of epilepsy. Thus, the Compression coated tablet formulations can be manipulated to release the drug exactly in a timely manner in accordance with Chrono therapeutic objectives.

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