



Comparative in vivo evaluation of marketed sustained release and optimized pulsatile formulation of propranolol hydrochloride

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ABSTRACT

Chrono pharmaceutical drug delivery system is devoted to the availability of active on a pace that idyllically matches biological requisite of disease therapy. It embodies time controlled and site-specific drug delivery systems, subsequently optimizing therapeutic action and lessening side-effects. In the present study, the optimized pulsatile formulation of Propranolol Hydrochloride ought to deliver a drug at the pre-set pattern at the right time and site is evaluated for pharmacodynamic and pharmacokinetic performance after oral administration and compared with an existing marketed sustained formulation of Propranolol HCl. The study was carried out in male New Zealand albino rabbits through the cross over design pattern and levels of plasma measured by means of LC-MS/MS method. Pharmacokinetic parameters of designed pulsatile formulation were measured and observed to have statistical significance with the existing marketed sustained formulation. The In-house pulsatile dosage form able to show the lag phase and the mean residence time of pulsatile dosage form (23.20.14h.) was found to be beyond the marketed sustained dosage form (14.8 0.01h.). Pharmacodynamic data revealed a maximum guard against adrenaline levels at 6 h post-oral intake and dropped to 50% after 12 h with Marketed formulation. Whereas in the case of pulsatile formulation administration, maximum protection was obtained at 12 h. and continued over a period of 18 h. It is concluded that the designed pulsatile formulation offers a promising way of drug release for a programmed time period at the desired site, once the administration time and pulse time are aligned with a circadian pattern.

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INTRODUCTION

Propranolol Hydrochloride is a synthetic β -adrenergic receptor blocker indicated in the management of hypertension, decrease angina frequency and other cardiovascular disorders. It is subjected to an extensive first-pass metabolism by the liver after taken orally and approximately 15 to 23% (Rao and Saikishore, 2015) of propranolol reach the systemic circulation. Its plasma half-life is from 3 to 6 hrs and rapidly eliminated, thus repeated administration desired for maintenance

of plasma levels (Malhotra, 2017). A number of functions of the heart undergo circadian patterns. Cardiac actions such as Blood pressure, stroke, ventricular arrhythmias, vascular tone and effective circulating volume occur with a circadian pattern that demands the availability of the administered drug in the systemic circulation to be high in the morning hours (Eddington *et al.*, 1998). Hence, an optimized pulsatile dosage form is designed to avoid first-pass hepatic metabolism, repeat administration and circadian combat rhythm (Krishan *et al.*, 2020). Therefore In the current study, the pharmacokinetic and pharmacodynamic parameters of In-house designed pulsatile formulation was evaluated and compared statistically with an existing marketed sustained formulation to reveal the relative bioavailability of drug at the desired time and duration (Bojja *et al.*, 2014).

MATERIALS AND METHODS

Reagents and chemicals

"The *in vivo* study of the optimized formulations were performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India Prior approval by Institutional animals ethics committee" (Rama *et al.*, 2017) was got for performance of experiments (Ref: BCOP/IAEC/VI-2/ 2018-2019, Dated 21-10-18). Marketed Propranolol hydrochloride SR formulation (PROLOG TR 80) and Insoluble capsular pulsatile formulation of Propranolol hydrochloride containing equal amounts of HPMC4: lactose plug prepared in the laboratory conditions. Eudragit FS 30D was obtained from Evonik AG, Darmstadt, Germany. HPMC K4, Cellulose acetate phthalate were procured from SD fine chemicals, Mumbai and remaining reagents used are of analytical- grade.

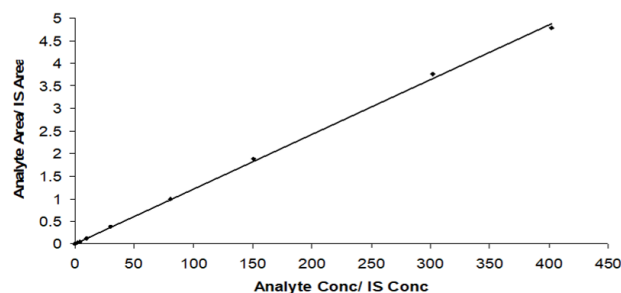


Figure 1: Calibration Curve for Estimation of Propranolol Hydrochloride in Plasma

Subject selection

Twelve New Zealand healthy rabbits bearing an average age of 10 ± 2 weeks and a mean weight of

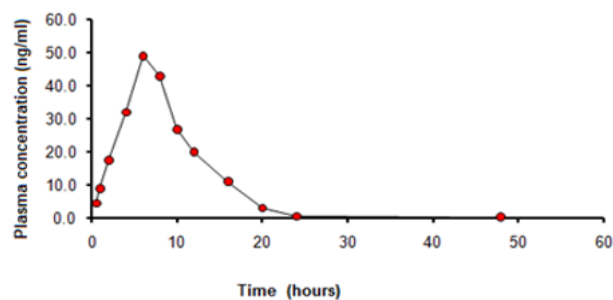


Figure 2: Plasma Concentration-Time Curve of Propranolol hydrochloride following oral administration of the marketed formulation

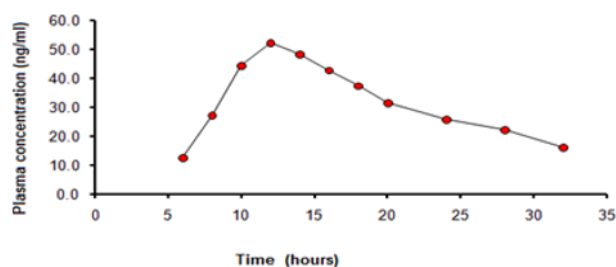


Figure 3: Plasma Concentration-Time Curve of Propranolol hydrochloride following oral administration of pulsatile formulations

3 ± 0.2 kg were used in this study. All the rabbits were separated into two groups, with six in each group and are overnight fastened. Both groups were accommodated in separate cages and thus taken care of no strain on the animals. Food and water were accessible ad libitum at the entire duration throughout the study (Krishna *et al.*, 2017). The study was conducted in a crossover design with two weeks of washout periods in between the two experiments (Rama *et al.*, 2017). The animal dose of Propranolol hydrochloride calculated pertinent to the human dose with the below-mentioned formula. The above dosage form was administered through gastric intubation method.

Human dose of Propranolol hydrochloride = 80 mg.

$$\text{Animal dose} = \frac{\text{Human dose} \times \text{Animal weight}}{\text{Human weight}}$$

$$= 80 \times 3 / 70 = 3.42 \text{ mg/kg.}$$

The dosage forms were administered through the gastric intubation method, as shown in Table 1.

Blood sampling

About 1 ml of blood samples collected from tracheal lobular vein of rabbit and subsequently stored blood in screw-top heparinized plastic tubes, the sampling intervals of blood is 0 mins (pre-dose), 1 hr, 2 hr, 4 hr, 6 hr, 8hr, 10 hr, 12 hr, 14 hr, 16 hr, 18 hr, 20 hr, 24hr and 48 hr" (Kishore and Murthy, 2011).

Table 1: Treatment parameters for oral and optimized pulsatile formulation administration of Propranolol hydrochloride

Treatment parameters	Propranolol hydrochloride	
	Oral solution (5ml)	Optimized pulsatile formulation
A dose of drug (mg /kg)	3.42	3.42
Treatment groups	Group I	Group II

Table 2: Statistical Treatment of Pharmacokinetic Parameters (Mean S.D.) following oral administration of marketed Sustained formulation and pulsatile formulations of Propranolol hydrochloride

Pharmacokinetic parameter	marketed formulation	pulsatile formulations	Calculated 't' value
C _{max} (ng/ml)	49.0±0.31	52.1±0.42	6.71***
MRT (h)	14.8±0.01	23.2±0.14	22.48***
t _{1/2} (h)	6.76±0.011	8.84±0.014	5.74***
K _{el} (h ⁻¹)	0.10±0.002	0.07±0.002	3.86***
K _a (h ⁻¹)	0.41±0.01	0.19±0.02	6.66***
AUC ₀₋ (ng h/ml)	356.2±1.43	951.7±2.07	56.59***

Null hypothesis (H₀): No significant variation observed between pharmacokinetic parameters of oral administration of marketed formulation and pulsatile formulations of Propranolol hydrochloride.

Table value of 't' with 10 DF at the 0.001 level is 4.587.

Result: H₀ is not accepted as the calculated 't' value is greater than the table value of 't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was a significant difference between the pharmacokinetic parameters of oral administration of marketed formulation and pulsatile formulations of Propranolol hydrochloride.

Values are presented in Mean SD (n = 6); *p<0.05, ** p<0.01,***p<0.001

Table 3: Percent reduction of ectopic beats after marketed sustained release and optimized pulsatile formulations administration of Propranolol hydrochloride in rabbits.

Formulation	Percent reduction of ectopic beats										
	1h	2h	3h	4h	6h	8h	10h	12h	14h	16h	18h
Marketed sustained release formulations	59.8±6.2	72.2±4.6	86.4±2.3	91.2±3.1	99.3±1.3	88.5±2.4	72.6±1.4	48.6±2.5	—	—	—
Optimized pulsatile formulations	—	—	—	—	56.2±4.3	77.4±4.2	89.6±3.8	98.2±4.8	87.6±3.8	75±4.8	49.6±3.8

The plasma was instantly parted by centrifugation for the duration of 5 mins, a speed of 4000 rpm and the sample was frozen at -20 °C till examined by LC-MS/MS method.

Determination of Pharmacokinetic Parameters

“Various pharmacokinetic parameters such as Area under the curve (AUC), Peak plasma concentration (C_{max}), Elimination rate constant (K_{el}), Time

at which peak occurred (T_{max}), Biological half-life (t_{1/2}) and mean residence time (MRT) were calculated using the non-compartmental pharmacokinetic data analysis software PK Solutions 2.0™ (Summit Research Services, Montrose, CO, USA). The pharmacokinetic parameters of the tested formulations were statistically analyzed using paired sample's t-test for normally distributed results of C_{max}, K_a, K_e, MRT and AUC_{0-α} value. All tests

were executed at 0.001 level of significance" (Sangalli *et al.*, 2001; Rao *et al.*, 2003).

Estimation of Propranolol hydrochloride in plasma (LC-MS/MS method)

Chromatographic conditions

"A summary of the chromatographic and mass spectrometric conditions is as follows:

HPLC : PERKIN ELMER

Mass : API 2000

Ion source : Heated Nebulizer

Polarity : Positive ion mode.

Detection ions

Propranolol : 260.200 amu (parent), 56.0 amu (product)

Metoprolol : 268.200 amu (parent), 116.20 amu (product)

Column : Vertiseq-BDS, 5 μ , 4.6x150mm, C18

Column oven temperature: 35.0 °C

Peltier temperature : 15 °C

Mobile phase : 0.1% Formic acid: methanol: acetonitrile (pH: 6.0 \pm 0.1) (10:45:45)

Flow rate : 1.000ml/min.

The volume of injection : 20 μ l

Retention times : Propranolol: 1.6 to 2.4 min

: Metoprolol: 1.5 to 2.3 min

Run time : 3.00 min" (Siluk *et al.*, 2007).

Preparation of propranolol hydrochloride stock solution

Propranolol hydrochloride working standard corresponding to 10mg Propranolol hydrochloride was dispensed into 10 ml volumetric flask, solubilized with methanol and volume makeup to 10ml was done with methanol. The concentration of resulting solutions was calculated by considering the purity of Propranolol hydrochloride. The resultant solution was labeled and stored at 5 \pm 3°C.

Preparation of metoprolol as an internal standard stock solution

Metoprolol working standard equivalent to 10 mg of metoprolol was dispensed into 10 ml volumetric flask, solubilized with methanol and volume makeup up to 10ml was done with methanol. The concentration of the resulting solution was calculated by taking into consideration the potency of metoprolol. The solution was labeled and stored in a cold room at 5 \pm 3°C. The stock solution was diluted through 60% methanol to achieve a concentration of about 1 μ g/ml.

Preparation of stock dilutions of standard propranolol hydrochloride solution

Stock dilution of Propranolol hydrochloride concentration between 40.240 ng/ml - 8047.32 ng/ml was prepared through 60% methanol by means of stock solution dilution prepared for the standard calibration curve. Concentrations of standard Propranolol hydrochloride stock dilutions were prepared.

Spiking of plasma for standard calibration curve

Propranolol hydrochloride Concentration limits from 2.012 ng/ml - 402.366 ng/ml were prepared by plasma, as shown in Figure 1 and were labeled as CC1 to CC8. The calibration curve standards were prepared fresh for each validation run. Concentrations of stock dilutions of standard Propranolol hydrochloride solution with plasma were prepared.

Sample preparation

Calibration curve standards, Blank and the subject samples were withdrawn out of the deep freezer and permitted them to thaw. To assure uniformity of the contents, thawed samples were vortexed. To 0.5 ml of plasma sample in vial, 50 μ l of Metoprolol (1 μ g/ml) was added. To plasma blank and pre-dose (0.0h), 50 μ l of 60% methanol was added. To assure uniformity of the contents, the samples were vortexed.

Approximately 3 ml of ethyl acetate solution was added and centrifuged for 10 min at approximately 4000 rpm at 20°C and supernatant (organic layer) dispensed into additional vial. The organic part was evaporated by purging nitrogen gas at 45°C. The deposit was reconstituted with a 0.25 ml reconstitution solution and vortexed. The samples are relocated into auto-injector vials and were loaded into autosampler. 20 μ l of the sample was loaded onto the LC-MS/MS system. Analytic Concentrations of stock dilutions of Propranolol hydrochloride standard were prepared with plasma.

Data processing

Chromatograms are obtained by usage of computer-based lab solution software, version 75.8, provided by Shimadzu Corporation. The concentration of unknown samples is measured from the calibration standard of spiked plasma through the equation of regression analysis by $+1/x^2$ as a weighting factor (Bhat *et al.*, 2011).

$y = mx + c$; Where,

y - Area of Propranolol hydrochloride peak / Area of Metoprolol peak (analyte / ISTD);

x - Propranolol hydrochloride concentration; c - Y intercept; m - Calibration curve slope.

Linear regression analysis equation of stock dilutions of standard Propranolol hydrochloride solution with plasma is $y = 0.0183x + 0.0024$

Pharmacodynamic evaluation of optimized Propranolol hydrochloride formulations

In animals, Pharmacodynamic factors resulting from the intake of oral and optimized pulsatile formulation of Propranolol hydrochloride was appraised by % β -blockade. The animals used for the study have admittance to take water; however, before the intake of drug formulation was fasted since 24. All the animals were sedated with urethane (1 g/kg, i.p.). With the subsequent recording of a normal electrocardiogram. Prior intake of drug formulations, isoprenaline (2 mg/kg, i.v.) in normal saline was directed into the marginal ear vein. Electrocardiogram was chronicled instantly and kept control. The animal dose of Propranolol hydrochloride and its microspheres was calculated pertinent to the human dose. Electrocardiogram was recorded after 1, 2, 4 and 6 h in case of oral and 4, 8, 12, 20 and 24 h in case of optimized pulsatile formulation post-administration, isoprenaline (2 mg/kg, i.v.) response was recorded as before. Each animal was used as its own control. The % β -blockade was obtained from the variance of response with isoprenaline at a time "0" and respective time interval after intake of the designed formulations.

RESULTS AND DISCUSSION

The *in vivo* experiments were conducted as per the protocol and procedure described earlier. The capability of adopted pulsatile capsules as a dosage form to discharge drugs at a programmed time was examined in a rabbit's post oral intake.

Bioanalytical procedures, used for the quantitative determination of actives and metabolites of active in biological fluids (plasma, serum, saliva, urine, etc.,) exhibit a vital role in the estimation and interpretation of pharmacokinetic data. For the successful conduct of the pharmacokinetic study, the design of specific bioanalytical methods plays an important role in the quantitative determination of actives and metabolites (analyte). The LC-MS/MS methods were highly sensitive and suitable for the detection of drug in plasma, even in low concentrations. The outcomes after oral intake of Propranolol hydrochloride specified the maximum plasma concentration (C_{max}) 49.00.31ng/ml at 6 h (t_{max}) while pulsatile formulation administration exhibited the maximum plasma concentration of 52.10.42 ng/ml at 12 h (t_{max}) after an initial lag time 5 h. Propranolol Hcl taken orally lead to relative variable

AUC of 356.21.43 ng.h/ml, whereas the pulsatile formulations resulted in AUC of 951.72.07 ng.h/ml. The mean residence time of pulsatile formulations administration (23.20.14h) was found to be more than oral administration (14.80.01h). The results were represented in Table 2.

The *in vivo* results indicated that the pharmacokinetic parameters significantly differed following pulsatile formulations compared to oral marketed sustained release formulation. Pharmacokinetic data revealed a maximum guard against adrenaline levels at 6 h post-oral intake and dropped to 50% after 12 h (Figure 2). while pulsatile formulation resulted in a maximum guard against adrenaline levels at 12 h and was prolonged over a period of 18 h. In line with Chrono modulated treatment of Blood Pressure, the lag time criteria of 5 hrs. & sustained delivery for 12 hrs. (Figure 3) duration was contented.

The percent reduction of ectopic beats after marketed sustained release formulation and pulsatile formulations of propranolol hydrochloride was represented in Table 3. Pharmacokinetic data revealed a maximum guard against adrenaline levels at 6 h post-oral intake and dropped to 50% after 12 h. while pulsatile formulation resulted in a maximum guard against adrenaline levels at 12 h and was prolonged over a period of 18 h.

CONCLUSIONS

The results revealed the capability of the adopted delivery system in delaying the discharge of drug for a predetermined time period and the likelihood of exploiting such delay to attain colon targeting. The dosage form can be taken at bedtime and will discharge the contents during early morning hours when the risk of hypertension is highest.

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