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IN VIVO EVALUATION OF MATRIX MINI-TABLETS OF BOSENTAN FOR CONTROLLED RELEASE

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ABSTRACT

A controlled release Pulsincap dosage form of Bosentan was developed for the treatment of pulmonary arterial hypertension (PAH), which lead to serious cardiovascular complications. The main objective of the present study is to enhance the bioavailability of oral controlled release by developed matrix mini-tablet formulations at a therapeutic dose containing 15.625mg of Bosentan in each mini-tablets. Four mini-tablets are equivalent to the dose of 62.5mg of Bosentan for 12 hours release. Various concentration of polymers such as Poly Ethylene Oxide, Eudragit RSPO, Guar Gum and Karaya gum were used for the development of matrix mini-tablets. Based on the results obtained after performing physicochemical characteristics, *In vitro* drug release and stability studies on all batches, among all formulations, MT16 containing Karaya gum at 40mg has shown best and satisfactory results. Hence, this formulation was considered as best and selected for further *In vivo* evaluations. The present study focuses only on in vivo studies performed on best formulation MT16. As the conclusion, it was obvious that the mini matrix formulations were able provide controlled release of Bosentan in Rabbits. Knowing that the Rabbits attained controlled release of drug, which was observed with the mini matrix tablets when compared to the control is expected to be even longer in humans. This would maximize absorption by allowing the slowly released drug in upper part of small intestine (i.e. the sight of absorption). The mini matrix tablets showed better bioavailability characteristic while comparing with a commercial conventional tablet containing 62.5 mg of Bosentan (Bosentas, Cipla Ltd., Mumbai) with higher mean $AUC_{0-\infty}$, C_{max} and longer T_{max}, however non significantly different.

Key Words:- Bosentan; matrix mini-tablets, Cmax, Tmax, Lambda_z, t½el.

INTRODUCTION

Many problems are associated with a conventional multiple-dosing regimen of long-acting therapy, such as systemic accumulation of the drug leading to side effects or toxicities, flip-flop profile of the plasma drug level, and poor patient compliance. The development of controlled release tablets had a clinical rational as it may reduce dose related side effects, improve efficacy and compliance to drug therapy. Controlled release products may be developed to reduce dose frequency, which adds to convenience of use, which in turn may facilitate compliance. Another rationale for developing controlled release preparation is smoothing the peaks of the plasma concentration curves (controlled release) in order to prevent peak concentration related adverse events (Eli Gabbay et al., 2007).

Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, least aseptic constraints and flexibility

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in the design of the dosage form. It is well known that controlled release dosage forms may offer one or more advantages over immediate release formulations of same drug (Hitesh P. Patel et al., 2011).

Hence, an attempt has been made on developing a controlled release matrix mini-tablets dosage form of Bosentan for the treatment of pulmonary arterial hypertension (PAH), which lead to serious cardiovascular complications. Bosentan is an oral medication classified as an endothelin receptor antagonist (ERA) which is approved for the treatment of pulmonary arterial hypertension (PAH) in World Health Organization (WHO) Group 1 patients. Bosentan works by blocking endothelin, a substance made by the body (Kathryn Teng, MD 2014). Endothelin causes blood vessels to narrow (constrict). It also causes abnormal growth of the muscle in the walls of the blood vessels in the lungs. This narrowing increases the pressure required to push the blood through the lungs to get oxygen. By blocking the action of endothelin, causing vessels to relax, Bosentan decreases the pulmonary blood pressure to the heart and improves its function. This generally results in the ability to be more active. Research studies have verified this improvement (Sajid Bashir et al., 2008).

Several controlled release formulations have been developed recently. Tablet based or capsule based formulation is the basis of the new drug delivery technology that addresses emerging trends and requirements (Blood collection, 2008).

Bioavailability is a pharmacokinetic term that describes the rate and extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of drug action. Since pharmacologic response is generally related to the concentration of drug at the receptor site, the availability of a drug from a dosage form is a critical element of a drug product's clinical efficacy. However, drug concentrations usually cannot be readily measured directly at the site of action. Therefore, most bioavailability studies involve the determination of drug concentration in the blood or urine. This is based on the premise that the drug at the site of action is in equilibrium with drug in the blood (Seham A. Elkheshen et al., 2004).

It is therefore possible to obtain an indirect measure of drug response by monitoring drug levels in the blood or urine. Thus, bioavailability is concerned with how quickly and how much of a drug appears in the blood after a specific dose is administered. The bioavailability of a drug product often determines the therapeutic efficacy of that product since it affects the onset, intensity and duration of therapeutic response of the drug (Guidance for Industry, 2001).

The main objective of the present study is to enhance the bioavailability of oral controlled release by developed matrix mini-tablet formulations at a therapeutic dose containing 15.625mg of Bosentan in each mini-tablets. Four mini-tablets are equivalent to the dose of 62.5mg of Bosentan for 12 hours release (Hsin-ya Leeand and Yung-jin Lee, 2010).

Various concentration of polymers such as Poly Ethylene Oxide, Eudragit RSPO, Guar Gum and Karaya gum were used for the development of matrix mini-tablets. Based on the results obtained after performing physicochemical characteristics, *In vitro* drug release and stability studies on all batches, among all formulations, MT16 containing Karaya gum at 40mg has shown best and satisfactory results. Hence, this formulation was considered as best and selected for further *In vivo* evaluations. The present study focuses only on in vivo studies performed on best formulation MT16 (Nattee Sirisuth and Natalie D. Eddington, 2009).

MATERIALS AND METHODS

Selection of Animals

Twelve healthy white albino rabbits of either sex ranging in body weight from 1-1.5 kg were used for both best formulations separately. All the animals were maintained under similar conditions. The animals were fed with fresh green fodder and black gram in the morning and evening, while water was provided freely as much they required.

Study Protocol

Administration of the two products (Test-MT16 and Reference-Bosentas, Cipla Ltd., Mumbai) to the animals was carried by means of a two-way crossover design. The models were randomly divided into two equal groups and assigned to one of the two sequence of administration. Each animal received a single dose of 0.62mg/kg of Bosentan as mini matrix tablet by oral for reference along with 15-20mL of water. The animal doses were calculated using US FDA online animal dose calculator (Oncology Tools, 2010). The mini matrix tablets were specially prepared according to body weight of the animals without changing the proportions of all ingredients including drug. The study was approved with registration no. 1450/PO/a/11/CPCSEA/dated: 23-01-2012 by the Institutional Animal Ethical Committee (IAEC), Gurram Balanarasaiah Institute of Pharmacy, Edulabad, Ghatesar, RR District, Andhra Pradesh and the animal study was performed at the same research centre.

Sampling procedure

The blood samples were collected through the ear marginal vein of the rabbits, which were held in wooden cages, in heparinized glass centrifuge tubes with the aid of sterilized disposable plastic syringes just before and at 0, 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24h after the drug administration. The blood samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma for analysis (Jaber Emami, 2006).

Quantitative drug analysis

The concentration of drug in plasma was determined by the high performance liquid chromatographic technique with ultraviolet detection at 272nm. Sample preparation procedure and few other variables were suitably adjusted in the lab. Estimation of drug concentration was carried out by interpolating the peak area of best formulation on a calibration curve of spiked the blank plasma over the range assayed (Selye, H, 1936).

Sample preparation

After separating the plasma from blood sample, an equal amount of 5% perchloric acid was added for protein precipitation, vortexed for two minutes then centrifuged at 2000 rpm for 10 minutes. The aliquot was separated for injecting into the HPLC system. All samples from a single animal were assayed on the same day to avoid inter-assay variation. The limit of Bosentan quantization in plasma was 5ng/mL (Hanson HM et al, 1960).

Pharmacokinetic and statistical analysis

The pharmacokinetic characteristics such as Cmax (ng/mL), Tmax (h), Lambda_z, t½el (h), Vd (ng/mL), Cl/F, AUC_{0-24} (ng.h/mL), AUC0-∞ (ng.h/mL), AUC ratio (%) and MRT0-∞ (h) of drug were determined from the plasma concentration time profile. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were obtained directly from the plasma concentration-time data. The area under the plasma concentration time curve up to the last time (t) showing a measurable concentration (C_t) of the analyte (AUC_{0-t}) was determined by applying the linear trapezoidal rule. Terminal half-life (t½λz) – a larger number means, compound disappears more slowly during the terminal (last, "lambda-z") phase. The $AUC_{0-\infty}$ values (express the magnitude of absorption) were determined by adding the quotient of $*C_t$ and the appropriate k_{el} to the corresponding $AUC_{0-t,}$ which is,

$$AUC_{0-\infty} = AUC_{0-t} + *C_t / K_{el}$$

Where $*C_t$ is the last detectable plasma concentration.

The sampling period covered more than 96% of the total AUCs for both reference and test. The apparent elimination half-life ($t_{1/2}$) of Bosentan in plasma was calculated by using the following equation,

$$t_{½} = (\ln 2) / K_{el}$$

The ratio of $C_{max}/AUC_{0-\infty}$ was also computed and used as a measure for the rate of absorption.

All values are expressed as the mean ± standard deviation (SD). The pharmacokinetic parameters obtained by following a single dose administration of the reference standard tablets and the floating tablets to normal Rabbits were compared using paired 't' test, considering a probability of $P<0.05$ to be significant (Chun-Chao Chang et al., 2005).

Bioavailability and statistical analysis were performed according to the FDA guidelines by using a software Bear v2.6.3 provided by Chunghwa Pharmaceutical Research Foundation (YJ), Kaohsiung & Taipei, Taiwan (Piper DW and Stiel DD, 1986). Results of Pharmacokinetic parameters are presented in Table 1, observed plasma drug concentration graphs are shown in Figure 1 to 3.

RESULTS AND DISCUSSION

The C_{max} was found to be 1544.500 ± 171.516 and 1762.167 ± 181.351 ng/mL for the reference standard and mini matrix tablets of MT16 respectively and the corresponding T_{max} were 1.667 and 10.333h. It is obvious that the values of C_{max} were very close for the two treatments and no significant difference were obtained between them. The mini matrix tablets of MT16 was exhibited delayed T_{max} however, non-significantly different from the standard product at $p<0.05$. Both the data of C_{max} and T_{max} values were comparable with reported values in the literature.

The values of the MRT, which was the non-compartmental analogue of $t_{½}$, were also parallel to those of $t_{½}$. The tested formulation MT16 showed a higher MRT of 11.994±0.407h than the reference standard of 2.314±0.501h.

Statistical differences were observed in the $AUC_{0-\infty}$ for the reference standard (18543.977±1656.865ng/mL and the mini matrix tablets of MT16 (22013.757±1540.410ng/mL). The relative bioavailabilities were observed 88% and 94% for MT16. It was concluded that based on the C_{max}, T_{max} and $AUC_{0-\infty}$ values that lie within the acceptance range of the FDA guidelines (80-125%).

However, the pharmacokinetic data presented here did not show extraordinary variability when compared with most published bioavailability studies. The matrix mini-tablets showed more controlled release

characteristics as compared with the reference standard, although failed to show significant difference. Results of Pharmacokinetic parameters are presented in Table 1, observed plasma drug concentration graphs are shown in Figure 3.

Table 1. Pharmacokinetic parameters of reference and test of MT16

Parameters	Reference (Bosentas, Cipla Ltd., Mumbai)			Test		
	Mean	± SD	CV%	Mean	± SD	CV%
C_{max} (ng/mL)	1544.500	171.516	11.105	1762.167	181.351	10.291
T_{max} (h)	1.667	0.033	0.682	10.333	0.816	7.902
Lambda_z	0.291	0.017	6.067	0.326	0.0527	16.148
$t_{½}$el (h)	0.382	0.145	6.075	2.164	0.320	14.790
V_d (ng/mL)	1.689	2.339	12.516	14.196	1.989	14.013
Cl/F	5.430	0.504	9.276	4.560	0.301	6.602
AUC_{0-24} (ng.h/mL)	18263.287	1654.054	9.057	21741.286	1493.499	6.869
$AUC_{0-\infty}$ (ng.h/mL)	18543.977	1656.865	8.935	22013.757	1540.410	6.997
AUC ratio (%)	98.477	0.180	0.183	98.769	0.268	0.272
MRT0-∞ (h)	2.314	0.501	4.067	11.994	0.407	3.394

Each value represents the mean ± standard deviation (n=6)

Figure 1. Plot showing observed drug plasma concentration of Bosentan - Test product in six subjects

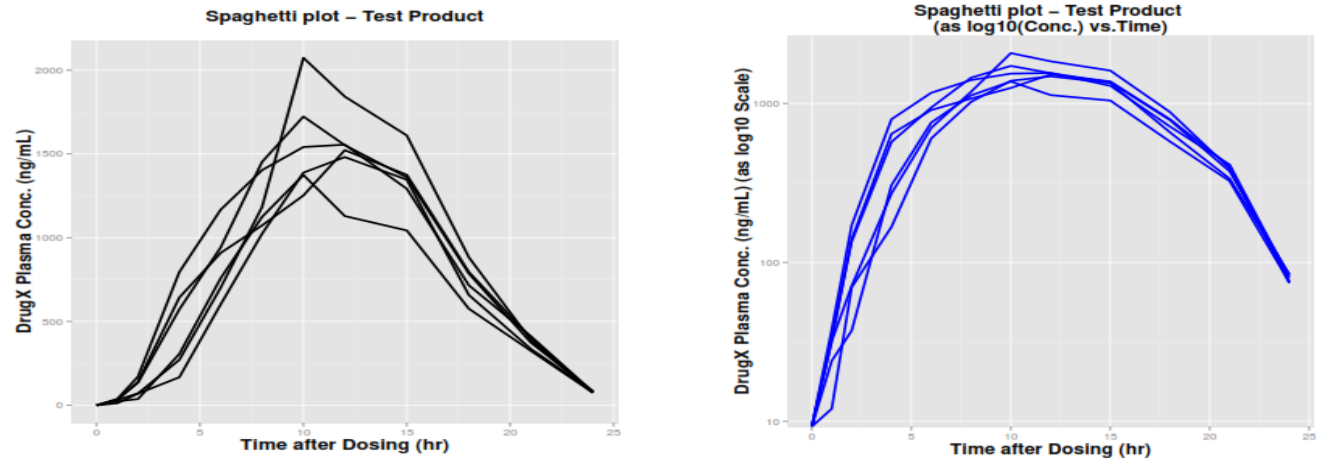


Figure 2. Plot showing observed drug plasma concentration of Bosentan - Reference product in six subjects

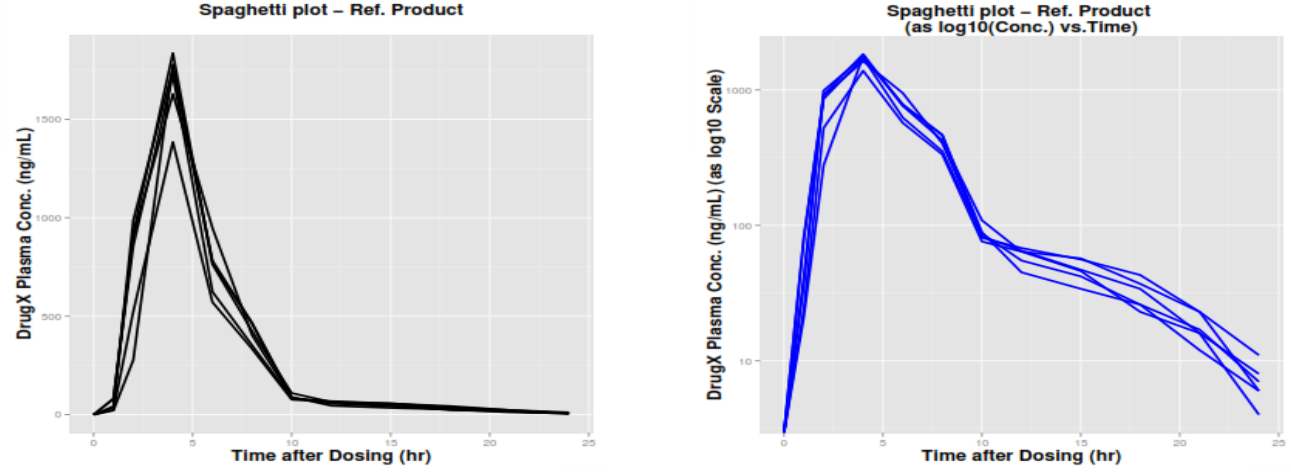
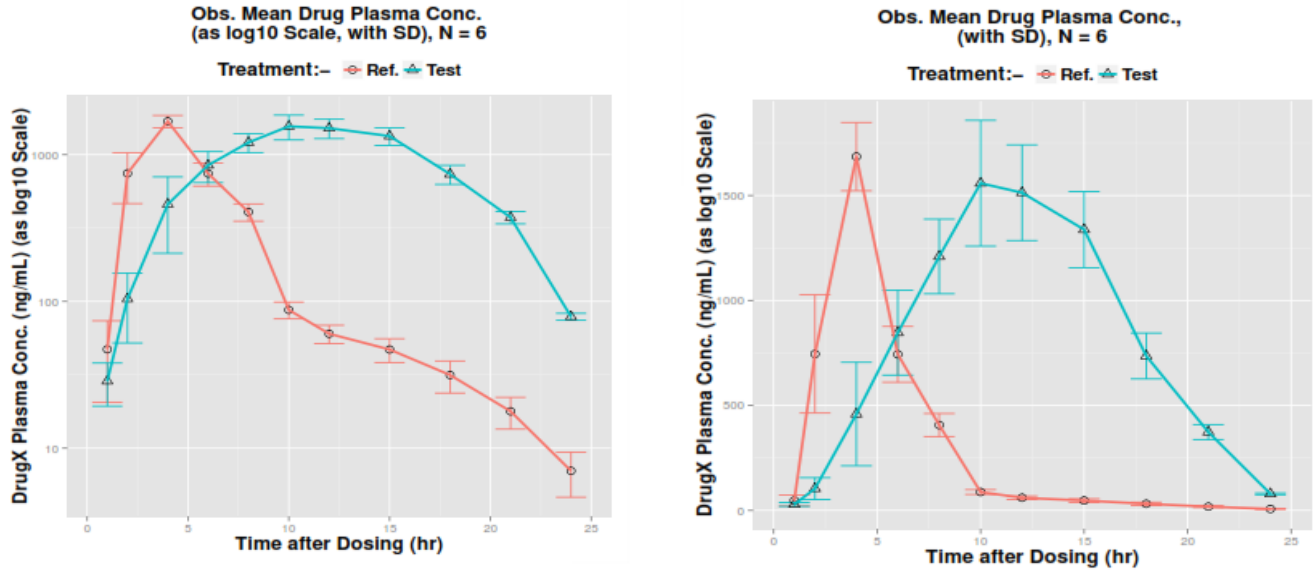


Figure 3. Observed mean drug plasma concentration with time profile and log[10] scale



CONCLUSION

As the conclusion, it was obvious that the mini matrix formulations were able provide controlled release of Bosentan in Rabbits. Knowing that the Rabbits attained controlled release of drug, which was observed with the mini matrix tablets when compared to the control is expected to be even longer in humans. This would maximize absorption by allowing the slowly released drug in upper part of small intestine (i.e. the sight of absorption). The mini matrix tablets showed better bioavailability characteristic while comparing with a commercial conventional tablet containing 62.5 mg of Bosentan (Bosentas, Cipla Ltd., Mumbai) with higher mean $AUC_{0-\infty}$, C_{max} and longer T_{max}, however non significantly different.

In present study the mini matrix tablets of Bosentan showed constant drug plasma concentration when compared with conventional dosage form. This may be due to its controlled drug release.

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