



**International Journal of Biological
&
Pharmaceutical Research**
Journal homepage: www.ijbpr.com

IJBPR

COMPARATIVE PHARMACOKINETIC PROFILE OF TINIDAZOLE COLON TARGETED FORMULATION WITH AN IMMEDIATE RELEASE FORMULATION

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ABSTRACT

The aim of the present investigation was to compare pharmacokinetic profile of Tinidazole colon targeted formulation with an immediate release formulation of Tinidazole. The formulated colon targeted tablet and a marketed immediate release tablet were subjected to pharmacokinetic (bioavailability) evaluation following an oral dose of 30 mg of drug per kg body weight of healthy rats. Several pharmacokinetic parameters like C_{max} , T_{max} , $AUC_{0 \rightarrow t}$, $AUC_{0 \rightarrow \infty}$, K_e and $T_{1/2}$ were determined from plasma concentration-time profile of all formulations. The absorption of Tinidazole marketed immediate release tablet and formulated colon targeted tablet resulted in 1.29 and 2.42 fold increase in bioavailability respectively as compared to pure Tinidazole drug administered as such. Results of these studies indicate that the formulated colon targeted tablet can be successfully used as a vehicle for enhancement of bioavailability of Tinidazole. Further the pharmacokinetic parameters indicated that the formulated colon targeted tablet exhibited prolonged effects of Tinidazole in rats.

Keywords: Tinidazole, colon targeted drug delivery, bioavailability, pharmacokinetic profile.

INTRODUCTION

Amoebiasis is an infection of large intestine caused by protozoan parasite, *Entamoeba histolytica*. Tinidazole and Metronidazole are drugs of choice in the treatment of amoebiasis, also effective against anaerobic microorganisms and are to be delivered to the colon for their effective action against trophozoites of *E. histolytica* that reside in lumen of the caecum, large intestine and adhere to colonic mucus and epithelial layers (Krishnaiah *et al.*, 2002). Tinidazole is used at doses of 2.0g/day for three days. From conventional formulation, Tinidazole is absorbed completely and

rapidly through an entero hepatic circulation and acts on trophozoites present in large intestine. For this process higher dose of about 2.0g of Tinidazole is required to achieve 4µg/ml concentration of the drug in the plasma. As conventional tablets are absorbed from the stomach, side effects like nausea, metallic taste, vomiting and head ache are observed. Therefore targeting the drug specifically to the colon is advantageous in treatment of amoebiasis (Sonali *et al.*, 2008).

Among the various approaches for targeting oral drug to colon, the system based on carriers that are degraded specifically by colonic bacteria was preferred. The natural polysaccharides remain undigested in stomach and small intestine and are degraded by the biodegradable enzymes present only in colon. So they are used as carriers

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to target the drugs to colon (Krishnaiah *et al.*, 2001). The natural polysaccharides like guar gum, pectin and dextrin were reported as good carriers individually and in combination for targeting the drug to colon (Chandramohan *et al.*, 2011).

The aim of the study was to evaluate the pharmacokinetic parameters of the formulated colon targeted tablets of Tinidazole in rat plasma and then to compare it with the pharmacokinetic parameters of immediate release tablets of Tinidazole.

MATERIALS AND METHODS

The study adheres to "Principles of Laboratory Animal Care" and is approved by the animal care committee IAEC/CPCSEA- Institutional animal ethics/Committee for the purpose of control and supervision of experiments on animals (TRCP/2011).

Healthy Wistar rats (both sex) of 150-250 \pm 10 grams were taken and grouped. Rats are divided into three groups (6rats/group). The rats were taken from Thanthai Roever college of Pharmacy animal house, which were quarantined a week before.

Drugs and Reagents

Tinidazole I.P was obtained as a gift sample from Cosmo Pharma, Chennai, India and natural polymers guar gum, pectin and dextrin were purchased from Otto, Mumbai. Other materials such as lactose, magnesium stearate, talc and polyvinyl pyrrolidone (PVP) K 30 were procured from Ranbaxy, India. HPLC grade acetonitrile from Rankem (Mumbai, India), water from Qualigens (Mumbai, India) were purchased. All other chemicals and reagents used in the study were of AR grade.

Preparation of colon targeted Tinidazole enteric coated matrix tablets

Colon targeted matrix tablets of Tinidazole were prepared by wet granulation technique. Lactose was used as diluent and the mixture of talc and magnesium stearate 1:1 ratio was used as lubricant. The matrix formulation containing 250mg Tinidazole with 125mg of pectin and 125mg of dextrin were formulated as given in table-1.

Tinidazole and all other ingredients were passed through sieve no 60 separately and mixed homogeneously. The dry powder blend was granulated with PVP K-30 which was dissolved in isopropyl alcohol. The coherent mass was passed through standard sieve no 22. The granules were dried at room temperature for 30minutes. The dried granules were again passed through sieve no 22 and were lubricated with a mixture of talc and magnesium stearate. Finally the lubricated granules were compressed into tablets

(Chandramohan *et al.*, 2011).

These tablets were enteric coated with 13% solution of Eudragit L-100 in isopropyl alcohol and water mixture (83:3) containing PEG 400 as plasticizer (at a concentration of 1.25 %w/w). After coating, the coated tablets are dried at 40°C for about 4 hours (Sinha *et al.*, 2002).

Characterization of formulated colon targeted formulation

Prepared tablet formulation was characterized for thickness and diameter, hardness, friability, uniform drug content, uniform weight and percentage weight increase. The *in vitro* release studies were carried out for the enteric coated tablets (Chandramohan *et al.*, 2011).

Instrumentation

The integrated HPLC (LC 20AT, Shimadzu corporation, Kyoto, Japan) was equipped with Rheodyne injector with 20 μ l loop, a SPD-M20A Prominence Diode array detector system. The separation of compounds was made on o Phenomenex – Luna, C18 at room temperature. The mobile phase was a mixture of phosphate buffer (pH 5.0)/ acetonitrile (75:25, v/v) pumped at a flow rate of 1.0ml/min and detection was carried at a wavelength of 295nm (Sethi, 1997).

Study design

The overnight fasted Wistar rats were divided into 3 groups each containing six animals. Group I animals received pure Tinidazole suspended in 0.5% carboxy methyl cellulose in distilled water given orally at a dose of 30 mg of the drug per kg body weight of animals. Group II animals received crushed granules of marketed Tinidazole tablet suspended in 0.5% carboxy methyl cellulose in distilled water given orally at a dose of 30mg of the drug per kg body weight of animals and Group III animals received formulated granules of colon targeted tablet of Tinidazole suspended in 0.5% carboxy methyl cellulose in distilled water given orally at a dose of 30 mg of drug per kg body weight of animals. Blood samples were collected at intervals of 1, 2, 4, 6, 8, 10, 12 and 15 hours after post dose into heparinized tubes from the orbital sinus of the animals. The plasma was separated immediately by using cold centrifuge at 3000 rpm for 15 minutes and the plasma was stored at - 4°C until analysis (Synder, *et al.*, 2008).

The samples stored in the freezer were brought to room temperature and the concentration of Tinidazole was determined by HPLC method. The chromatograms were recorded and the concentration of Tinidazole was determined from the ratio of area under curve of Tinidazole for each plasma sample using the standard curve.

Preparation of standard solution

Standard stock solution of Tinidazole was prepared in mobile phase of concentration of 1mg/ ml. The stock solutions were diluted to obtain working standard solution of concentration of 6µg/ ml to 30µg/ ml. The resulting solutions were centrifuged at 10000 rpm for 10min. After centrifugation the supernatant was separated and injected into the HPLC system. Standard curves were obtained by the least square regression analysis of drug peak area ratio as a function of theoretical concentration (Pasha *et al.*, 2010).

Preparation of sample solutions

To 100µl of plasma, 250µl of acetonitrile was added and mixed for a minute. To this 650µl of the diluent was added to make up the volume to 1 ml. The resulting solution was vortexed for 60 seconds and centrifuged at 10000 rpm for 10 minutes. The supernatant layer was separated and injected in to the HPLC system.

The concentration of the Tinidazole present in the plasma sample was calculated from the area ratio of the sample and standard using the calibration curve. The blank plasma sample was also analyzed prior to the analysis of Tinidazole standard preparation. No interface from the blank plasma was observed in the analysis of drug. The peaks were well resolved and the amount of Tinidazole present in the plasma was determined from the linearity curve.

Pharmacokinetic data analysis

The plasma concentration of Tinidazole at different time intervals were subjected to pharmacokinetic analysis to calculate parameters like maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under plasma concentration-time curve ($AUC_{0-\infty}$ and AUC_{0-t}), Elimination half life ($T_{1/2}$), Elimination rate constant (K_e), and Area under first moment curve $AUMC_{0-t}$.

The values of C_{max} and T_{max} were obtained directly from the concentration time curve data of Tinidazole. The area under concentration time curve from time zero to time of last quantifiable concentration was calculated using the linear trapezoidal method. The $AUC_{0-\infty}$ was calculated by using the equation:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$$

Where C_t is the last measurable concentration and elimination rate constant K_e was determined from the slope of the linear portion of graph plotted between logarithm of plasma concentration and time. $T_{1/2}$ was determined using the equation:

$$T_{1/2} = 0.693/K_e$$

The relative bioavailability of Tinidazole colon targeted tablet and immediate release tablets versus the pure Tinidazole were calculated as follows:

$$F\% = (AUC_{sample} / AUC_{oral}) \times (Dose_{oral} / Dose_{sample}) \times 100$$

RESULTS AND DISCUSSION

Colon targeted formulations of Tinidazole was successfully developed and characterized in terms of physicochemical parameters, uniform drug content, *in vitro* release and release kinetics (Chandramohan *et al.*, 2011). Plasma concentrations of Tinidazole at different time intervals were determined by reported HPLC method (Pasha *et al.*, 2010). The graph between plasma Tinidazole concentration and time was plotted for each formulation (Figure 1). Tinidazole was detectable in plasma within 60 minutes after its oral administration in rats. The absorption was rapid with pure Tinidazole and marketed immediate release tablet as indicated by low T_{max} value (2 hrs); whereas the colon targeted composition exhibited delayed absorption as demonstrated by high T_{max} (4hrs) values. This delayed absorption may be due to the extended release effect of the swelling polymers present in the matrix tablets which might have increased the viscosity and hence reduced the absorption rate. The C_{max} of the prepared colon targeted tablet was lower compared to pure Tinidazole and marketed immediate release Tinidazole tablets. The half-life of pure Tinidazole and marketed formulation were found to be less (6.66 and 8.885 hrs respectively), which specifies the rapid removal of drug from plasma and the rapid elimination of pure drug was further supported by high elimination rate constant (0.104 and 0.078 hrs^{-1}). On the contrary, the formulated colon targeted composition exhibited high half-life (28.875hrs), and low elimination rate constant values (0.024 hrs^{-1}) indicating that drug remains in the body for a long period of time and exhibits prolonged effect. The low value of AUC observed with pure Tinidazole and immediate release Tinidazole tablets may be due to the rapid absorption and elimination from the body. On the contrary, the formulated colon targeted Tinidazole tablets showed high AUC values indicating increased bioavailability of drug (Table 2). The smooth and extended absorption phase coupled with maintenance of plasma concentration for longer duration after administration in matrix tablets suggests reduced chance of dose dependent side effects of the drug. Further, all the parameters clearly reveal that the formulated colon targeted formulation exhibited prolonged effect of Tinidazole in rats.

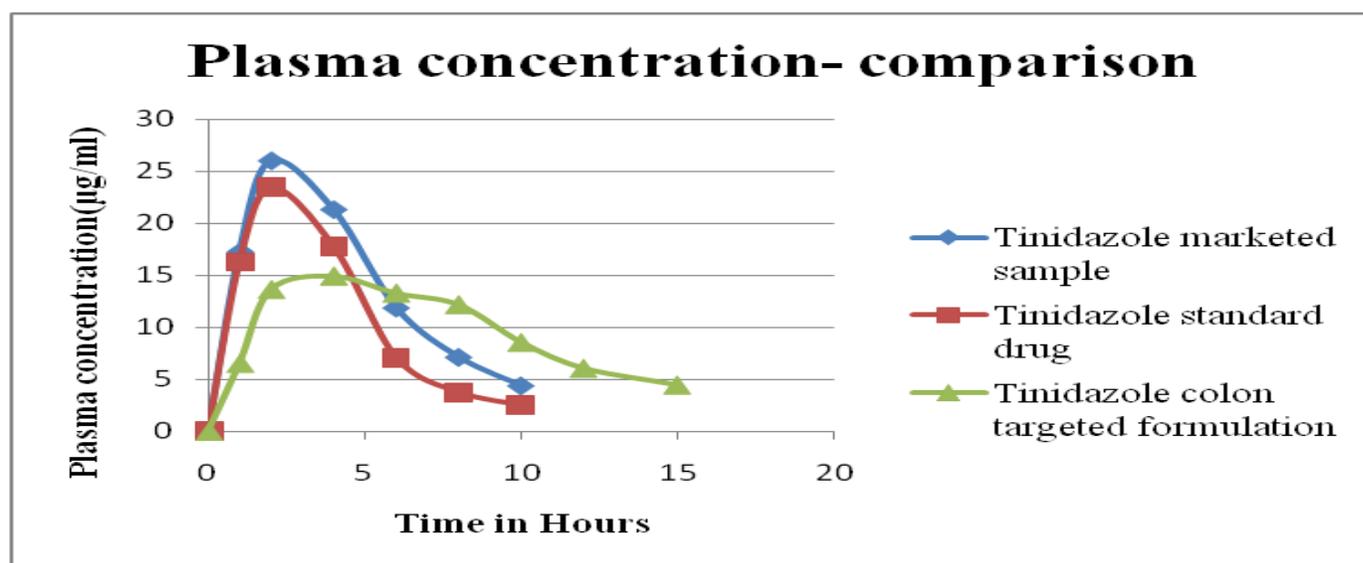
Table 1. Composition of colon targeted matrix tablet of Tinidazole

Ingredients	ETF6 (mg)
Tinidazole	250
Pectin	125
Dextrin	125
PVP K-30	15
Talc	5
Magnesium stearate	5
Lactose	125
IPA	q.s

Table 2. Pharmacokinetic parameters from the plasma concentration-time curve

S.No	Parameters	Tinidazole pure drug	Tinidazole marketed sample (MS1)	Tinidazole colon targeted formulation (ETF6)
1	C_{max} ($\mu\text{g/ml}$)	23.44	26.04	14.92
2	T_{max} (hrs)	2	2	4
3	K_e (hrs^{-1})	0.104	0.078	0.024
4	$T_{1/2}$ (hrs)	6.66	8.885	28.875
5	AUC_{0-t} ($\mu\text{g-hr/ml}$)	96	124	232.32
6	$AUC_{0-\infty}$ ($\mu\text{g-hr/ml}$)	332	475	1172
7	$AUMC_{0-t}$ ($\mu\text{g-hr}^2/\text{ml}$)	192.15	252.21	857.32

C_{max} = Maximum plasma concentration; T_{max} = time for maximum plasma concentration; K_e = elimination rate constant; $T_{1/2}$ = biological half life ; AUC_{0-t} = area under curve for 0 to T hours; $AUC_{0-\infty}$ area under curve for 0 to ∞ hours; $AUMC_{0-t}$ area under first moment curve for 0 to T hours

Figure 1. Plasma concentration time profile curve of various Tinidazole formulations**CONCLUSION**

The above study reveals that greater extent of absorption of prepared colon targeted formulation ETF6 than the immediate release marketed sample MS1 and pure Tinidazole. The absorption of Tinidazole from MS1 and

ETF6 resulted in 1.29 and 2.42 fold increases in bioavailability as compared to the pure Tinidazole sample. Results of these studies indicate that the formulated colon targeted tablet can exhibit a prolonged release of Tinidazole.

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