In vivo study of pH Dependent and Enzymatically Triggered Colon Targeted Tinidazole Microspheres

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ABSTRACT

Introduction: Shellac coated Tinidazole (TNZ) loaded pectin microspheres with the potential for colon targeted delivery has been evaluated to study in-vivo behavior. Materials and Methods: Biodistribution and pharmacokinetic studies were performed for the determination of concentration of TNZ in rats. This method was used to evaluate the colon targeting property of TNZ loaded microspheres. Pure TNZ or TNZ microspheres were given to rats by oral administration. Plasma and the different parts of gastrointestinal (GI) tract were taken after 2, 8, 12 and 24 h of oral administration of pure TNZ or TNZ microspheres to rats and the concentration of TNZ was measured. Results and Conclusion: Results obtained shows that pure TNZ distributes mainly in stomach and in low concentration to small intestine and colon. However, TNZ released from microspheres mainly distributes in colon. Therefore, this approach suggests that shellac coated TNZ loaded pectin microspheres has a good colon targeting property.

Key words: Biodistribution study, Colon targeting, Pharmacokinetic study, Pectin microspheres, Tinidazole.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the desired site in the body and also to achieve and maintain the required plasma concentration of the drug for a particular period of time. However, incomplete release of the drug and shorter residence time of dosage forms in the upper GIT lead to lower oral bioavailability. Such limitations of the conventional dosage forms have paved way to an era of controlled and novel drug delivery systems. The colon is a site where both local and systemic delivery of drugs can take place. Treatment could be made more effective, if it were possible for drugs to be targeted directly on the colon.

Oral colon-targeted drug-delivery systems have recently gained importance for delivering a variety of therapeutic agents for both local and systemic administration. Targeted delivery of drugs to colon has the potential for local treatment of a variety of colonic diseases such as irritable bowel syndrome (IBS), colorectal cancer and inflammatory bowel diseases (IBD) that includes both ulcerative colitis and Crohn’s disease. Apart from this local treatment, colon is used for the systemic absorption of proteins and peptides because of the less hydrolytic hostile environment in comparison with stomach and small intestine as well as the existence of specific transporters. Also colon is a good site for those drugs where a delay in drug absorption is required from therapeutic point of view e.g. in case of nocturnal asthma, arthritis, cardiac arrhythmias which are effected by circadian biorhythms. Additionally, the colon is a highly responsive site for the absorption of poorly absorbable drugs. By this colon targeted drug delivery it is also possible to prevent the side effects of drugs on healthy tissues and enhancement of drug uptake by targeted cells.

The approaches used in achieving colonic delivery of drugs include the use of prodrugs, pH-sensitive polymer coating, and time-dependent formulations. In addition, the use of biodegradable polymers such as azo-polymer and polysaccharide (eg, pectin and dextrin) for colon targeting are also reported in the literature. Among the different approaches to achieve colon-selective drug delivery, the use of polymers, specifically biodegraded by colonic bacteria, holds great promise. The pH-dependent systems exploit
the generally accepted view that pH of the human GI tract increases progressively from the stomach (pH 2-3) to the small intestine (pH 6.5-7.0) to the colon (7.0-8.0). In the present study, biodistribution and pharmacokinetic studies was performed to study in vivo characteristic of prepared colon targeted tinidazole microspheres. Tinidazole (TNZ) microspheres were prepared using natural polysaccharides (pectin) and pH-sensitive polymer (Shellac). This system is anticipated to protect the drug loss in the upper GI tract, which results from the inherent property of Shellac, and deliver TNZ in the colon only. The use of shellac as protective coating on the microspheres makes them able to release the drug at the particular pH of colonic fluid. A combined mechanism of release is proposed, which combines specific biodegradability of polymer and pH-dependent drug release from the coated microspheres.

MATERIALS AND METHODS

Materials

The drug, Tinidazole (TNZ) was purchased from Mundi Pharma, Merrut, India. Pectin and shellac was obtained from HiMedia Laboratories Ltd, Mumbai, India. Acetone, ethanol, n-hexane, and light liquid paraffin were purchased from Qualigens Fine Chemicals, Mumbai. Methanol, Acetonitrile and Span 80 was obtained from S. D. Fine Chemicals, Mumbai. All other chemicals were of analytical grade and were used as received. De-ionized double-distilled water was used throughout the study.

The in vivo study was performed in accordance with the protocol approved by the Institutional Animals Ethical Committee of Integral University, Lucknow, India, following the guidelines approved by the (IU/Pharm/Ph.D/CPCSEA/10/21).

Fabrication of Tinidazole (TNZ) loaded pectin microspheres

Pectin microspheres were prepared by emulsion cross-linking method. Pectin dissolved in 20 ml of distilled water and uniform solution was prepared. Dispersion of Tinidazole (TNZ), prepared by dispersing TNZ in 10 ml of dichloromethane, was added to the uniform polymeric solution with stirring. To produce an emulsion aqueous polymeric solution containing drug molecules was dispersed in 40 ml of light liquid paraffin containing span 80 (1.25%w/v) and stirred at 1000 rpm continuously to obtain stable w/o emulsion. The solution was rapidly cooled to 15°C by placing the beaker in an ice bath. After 20 min of stirring 10 ml of 1.3% w/v cacl₂ was added gradually to the system and stirred for 1 hr (allows the time for cross-linking). Resultant microspheres was filtered and washed with n-hexane and then dried. These pectin microspheres were microencapsulated by emulsion–solvent evaporation technique. Microspheres (100 mg) were suspended in 20 ml of coating solution prepared by dissolution of shellac (500 mg) in ethanol–acetone mixture and then emulsified into 40 ml of light liquid paraffin containing span 80. The emulsification process was carried out for 2 h at 1000 rpm with mechanical stirrer. The Shellac coated microspheres were collected and rinsed with n-hexane and dried. Prepared microspheres were evaluated for following in-vivo characteristics:

Study design

Albino male wistar rats of weight between 150-200 g were selected for in vivo studies, which was approved by the university’s committee on the ethical treatment of animals. Animals were kept in well-spaced ventilated cages, and maintained on a normal diet (grams soaked in water). The animals were divided into 3 groups of 4 animals each. The treatments were given by normal swallowing followed by water. The optimized formulation was selected in order to study in vivo performance of the preparation, on the basis of particle size, entrapment efficiency and in vitro release studies. Each group received one of the three treatments:

- First group: Served as controls
- Second group: Received the plain TNZ suspension, which was prepared using 1% gum acacia (dose calculated in relation to body weight of the animal).
- Third group: Given the formulation of TNZ microspheres.

In vivo Biodistribution Study

After 2, 8, 12 and 24 hrs, the animals were sacrificed and stomach, small intestine and colon were isolated. These segments of GIT were homogenized in phosphate buffer (pH 7.4 at 4°C) with a ratio of 1:10 gm/ ml using hand held homogenizer and then centrifuged at 10,000 rpm for 5 min at 4°C and supernatant was separated. In the separated supernatant, 1ml of acetonitrile was added and kept for 30 min and filtered. Supernatants were dried under a stream of nitrogen and redissolved in 0.1 ml of mobile phase. 0.02 ml of which was subjected to HPLC analysis. The drug content at different parts of GIT at different time intervals was calculated.
Pharmacokinetic study

Five ml blood samples were collected in the heparinized evacuated centrifuge tubes. Samples were collected by retro-orbital puncture at 2, 8, 12, and 24 hrs from the time of administration. The heparinized whole blood was centrifuged at 10,000 rpm for 5 min. 0.5 ml of supernatant plasma was taken and added 1 ml of methanol and agitated for 2 min. 0.02 ml supernatants were taken for HPLC analysis.\textsuperscript{10,11}

Statistical Analysis

Considering the inequality of variances between groups, a non-parametric method “Mann–Whitney U test” was adopted to analyze the average differences of the drug concentrations and the plasma concentrations in different groups, respectively.

Data Analysis of Pharmacokinetic study

Data was generated by assuming the first order absorption and one compartment model with first order elimination. The maximum peak concentration ($C_{\text{max}}$) and time of its occurrence ($T_{\text{max}}$) was directly computed from the plasma concentration vs. time plot. The elimination rate constant (Kel) was determined from the terminal phase of the log plasma concentration vs. time profile by least square regression analysis. From this Kel was calculated as $ Kel =$ slope X 2.303. The elimination half-life was calculated as $ t_{1/2} = 0.693 / \text{Kel}$. The area under the plasma concentration time curve from 0–$t$ ($AUC_{0-t}$) and from 0–$\infty$ ($AUC_{0-\infty}$), area under first moment curve from 0–$t$ ($AUMC_{0-t}$) and from 0–$\infty$ ($AUMC_{0-\infty}$) and mean residence time (MRT) was calculated using trapezoidal rule.\textsuperscript{8,10,11}

RESULTS

Biodistribution study

After the administration of pure TNZ, TNZ was observed to distribute in mucosa of the stomach and small intestine, while much less in the colon, with a maximal TNZ per gram of mucosa of 0.56 µg (Table 1). Its distribution decreased along gastrointestinal tract. By contrast, after the administration of TNZ microsphere, TNZ was not detected in the mucosa of the stomach and small intestine, while in the mucosa of colon TNZ was observed to be in a high concentration, with a maximal TNZ per gram of mucosa of 7.41 µg (Table 1). In colon mucosa, the amount of TNZ released from TNZ microsphere was much higher (Figure 1 and 2) than the amount of TNZ released from the pure TNZ.

Statistical results

The results of Mann–Whitney U test suggested that there were significant differences (P<0.05) between the TNZ concentrations at the same site and same time.

Pharmacokinetic study

After the administration of pure TNZ, TNZ was absorbed rapidly to enter the blood circulation. Its blood concentration peak was present at 2 hrs after administration, and was about 12.54 µg/ml. By contrast, after the administration of TNZ microsphere, the absorption rate of TNZ was decreased and blood concentration peak was present at 12 hrs after administration, and was about 2.16 µg/ml. Figure 3 shows plasma levels of TNZ after the oral administration of TNZ microsphere (equivalent to 27 mg/

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>Isolated organs</th>
<th>No. of animals</th>
<th>Sample time (in hrs)</th>
<th>Pure TNZ</th>
<th>TNZ Microsphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Stomach</td>
<td>4</td>
<td>2</td>
<td>14.53 ± 1.6</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>24</td>
<td>1.46 ± 0.21</td>
<td>0</td>
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<tr>
<td></td>
<td>Small Intestine</td>
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<td>6.24 ± 0.04</td>
<td>0</td>
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<td></td>
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<td>8</td>
<td>3.15 ± 0.19</td>
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<td>24</td>
<td>1.16 ± 0.04</td>
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<tr>
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<td>Colon</td>
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<td>2</td>
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<td>0</td>
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<td></td>
<td></td>
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<td>0.19 ± 0.01</td>
<td>1.96 ± 0.13</td>
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</tbody>
</table>

*Values are average of 3 readings ± standard deviation.
Figure 1: TNZ content (µg/g) in mucosa of different parts of rat GIT after oral administration of Pure TNZ.

Figure 2: TNZ content (µg/g) in mucosa of different parts of rat GIT after oral administration of TNZ Microsphere.

Figure 3: Plasma levels of TNZ after oral administration of Pure TNZ (27 mg/kg) and TNZ Microsphere (equivalent to 27 mg/kg TNZ) to rats.

Values are average of 3 readings ± standard deviation.
kg TNZ) and pure TNZ. The relative bioavailability (BA) of TNZ from the TNZ microsphere is 36.30% by comparing the AUC under the assumption that the bioavailability of TNZ from pure TNZ is 100% (Table 2). Results of various pharmacokinetic parameters are shown in Table 3.

**Statistical results**

After the administration of pure TNZ and TNZ microspheres, there were significant differences (P<0.05) between the blood concentrations at the same time.

**DISCUSSION**

In the present study, the detection method of the exact amount of pH dependent and enzyme-dependant TNZ microspheres in rats and its colon targeting property were explored. The findings suggest that the detection method adopted in this study is precise and reliable so that it can meet the needs of detection in vivo. Colon targeted TNZ microsphere has to survive passage through stomach and small intestine, to reach colon and to be degraded by enzymes of colonic microflora. In vivo study, the absorption behavior of TNZ was investigated to confirm the site-specific delivery of TNZ microspheres to the colon. The administration of plain TNZ gave a rapid increase in plasma TNZ levels and the time required to reach the maximum drug level, \( t_{\text{max}} = 2 \text{ h} \). In the case of the microspheres, on the other hand, the time required to reach the maximum drug level, \( t_{\text{max}} = 12 \text{ h} \). These results indicated that microspheres release TNZ site-specifically in the colon, therefore working as a colon targeted microspheres.

**CONCLUSION**

Targeting of drugs to specific sites of action provides several advantages over non-targeted drugs. These include the prevention of side effects of drugs on healthy tissues and enhancement of drug uptake by targeted cells. BA of TNZ was low after administration of TNZ microspheres because therapeutic effects depend on local concentrations of TNZ in the colonic mucosa, whereas systemic drug exposure might be one determinant of side effects. The side effects of TNZ are relevant to plasma levels of TNZ. Following administration of TNZ microspheres, lower plasma concentrations and higher delivery into the colon can be observed in comparison to administration of Plain TNZ.

The present results suggest that pH dependent & enzyme-dependant TNZ microsphere with shellac & pectin as a carrier has a good colon targeting property.

**REFERENCES**