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Development of microbial trigger based oral formulation of Tinidazole and its Gamma Scintigraphy Evaluation: A promising tool against anaerobic microbes associated GI problems

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ABSTRACT

Tinidazole is a versatile anti-amoebic and anti-anaerobic drug used in treatment of intestinal infection. The aim of present study was to develop and evaluate a guar gum based novel target release Tinidazole matrix tablet in animal models and healthy human volunteer using Gamma Scintigraphy technique. Anti-anaerobic and anti-protozoal activity of the developed formulation was studied in vitro against *Bacteroides fragilis* and *Dentamoeba fragilis*. Tinidazole was successful radiolabelled with ^{99m}Tc-pertechnetate using stannous chloride as a reducing agent and stable up to 24 h in normal saline and serum. Radiolabeled formulation was evaluated in 6 Newzealand white rabbits by gamma Scintigraphy in static manner up to 24 h for its retention in gastrointestinal tract (GIT). Similar set of study was conducted in 12 healthy human volunteers for similar objective Scintigraphy images of healthy human volunteer showed retention of optimized formulations in stomach up to 60 min, from where it moved to duodenum further and reached ileum in around 5 h. However, initiation of drug release was observed from intestine at 7 h. Complete dissociation and release of drug was observed at 24 h in colon due to anaerobic microbial rich environment. Results drawn from Scintigraphy images indicate that radiolabeled ^{99m}Tc-Tinidazole tablet transit through upper part of GI without disintegration. Hence the developed matrix tablet may have a role in treatment of intestinal infection caused by anaerobic bacteria.

1. Introduction

The application of naturally occurring hydrophilic biocompatible polymeric materials has been focused in latest researches for designing of oral controlled release formulation (Gande and Rao, 2011). Natural gums are hydrophilic polymers and are among the most popular because of their regulatory acceptance and cost effectiveness (Varshosaz et al., 2006). The use of naturally occurring plant-based pharmaceutical excipients has become important in the development of controlled release dosage forms, because of its ability to fabricate a vast range of material based on their properties and molecular weight (Perepelkin, 2005). Plant based materials can be customized to meet the demand of drug delivery systems and thus can compete with various synthetic excipients available in global market. Guar gum is water soluble thickening agents which has not been much studied for their pharmaceutical applications (McChesney et al., 2007). Tinidazole are the drug of choice in the treatment of amoebiasis and against the anaerobic microbial infection (Üner and Altinkurt, 2004; Calabresi and Chabner, 1996). The colon targeted use of tinidazole with guar gum based colon targeted formulations may not be uncommon. It was observed that the use of guar gum as a colon specific drug delivery carrier is based on its degradation by the bacteria present in the colon (Dakia et al., 2008). The colon is rich in anaerobic bacteria (Simon and Gorbach, 1984). Guar gum in the form of either compression coat over the drug or matrix tablet, its core might be degraded to a larger extent by the action of anaerobe's population of large intestine (Salyers et al., 1978). Since the tinidazole are active against anaerobic bacteria. (Alou et al., 2009) the usefulness of guar gum on concomitant administration of the drug with guar gum based formulation, in providing colon specific drug delivery is not well known. Most of the researcher developed the drug absorbing in upper part of intestine it may cause the un-utilization of drug in case of site specific drug. Guar gum based drugs can be sustained up to 5 days in colon (Philip and Philip, 2010). Oral drug delivery has been known for decades as the most widely utilized route of administration compared to all the routes that have been used for the systemic delivery of drug via various pharmaceutical products of various dosage forms. The cause that the oral route achieved such popularity is may be in part attributed to its ease of administration and ensured that by oral administration the drug is well absorbed. All the pharmaceutical products formulated for systemic delivery through oral route of administration irrespective of the mode of delivery (immediate, sustained or controlled release) and the modulation in dosage forms (either liquid or solid dispersion) should be developed within

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the intrinsic characteristics of pharmacokinetics, GI physiology, pharmacodynamics and formulation development is essential to achieve a systemic approach to the development of an oral pharmaceutical dosage form successfully. Sustained release system includes any delivery system that achieves release of drug over an increased period of time. Controlled release system considered as a system that maintains constant drug level in the blood at target time. If it is unsuccessful at this but nevertheless extends the period of action over that achieved by conventional delivery, it is considered a prolonged release system. Drug modification and dosage form have been investigated to control the time course of drug release and to maintain constant drug bioavailability. Sustained release oral dosage form has been demonstrated to enhance therapeutic efficiency by maintenance of a steady drug plasma concentration (Turner et al., 2004). Moreover, there are many advantages of sustained release dosage forms such as minimization of patient compliance problems, reduction of both local and systemic side effects, avoidance of night time dosing and minimization of "peaks" and "valleys" in drug blood vessels. With drugs of short half-life that require frequent dosing to maintain constant therapeutic levels, number of dose administrations can be reduced.

Above mentioned, motive behind developing this formulation is to develop a formulation against constipation caused by anaerobic bacteria (Pimentel et al., 2006) and related G.I. problems caused at high altitude. At high altitude the atmospheric pressure falls down, due to which in human G.I.T. occurs a translocation of bacteria and other microorganisms from large intestine to the small intestine and upper G.I. tract (Hamad and Travis, 2014). This causes an imbalance in the gut micro flora and thus disturbs the normal physiological activity of digestive system. Pathogenic microbes can grow and thus create problems. Small intestine bacterial overgrowth (SIBO) occurs in this case. Tinidazole is a drug with antiprotozoal, antibacterial activity which can kill various pathogenic microbes in the G.I. tract and prevent infections, and it can be achieved with the help of polysaccharide based intestinal delivery as the polysaccharide approach which is based on presence of microbial flora in the intestine which produce enzymes to degrade the polymer to release the drug. Therefore, this approach can preferably be used over other approaches because, in this approach the microbes present in whichever part of intestine will certainly release enzymes to degrade the polymer (polysaccharide) and thereby drug will be released, which in turn will act on those microbes and kill them so as to treat overgrowth, and it would be able to overcome the problem.

2. Material and Method

2.1. Materials

Tinidazole was purchased from M/s Hindustan Chemicals and Pharmaceuticals, Mumbai, India. Acacia gum, Tragacanth gum, Guar gum (viscosity of 1% aqueous dispersion is 3725 cps; particle size of 0.75 mm) was obtained from Merck specialties Pvt. Ltd. Mumbai, India. Dextrin and micocrystelline cellulose purchased from CDH fine chemical New Delhi India. Magnesium stearate, poly vinyl pyrollidine, Talc, and Aerosil purchased from High Purity Laboratory chemical, Mumbai, India. Thioglycollate medium, Hi-media, Mumbai, India. Acetone, isopropyl alcohol purchased from Merck specialties Pvt ltd, Mumbai, India and ^{99m}Tc-pertechnetate was procured through BRIT-INMAS. All other solvents & chemicals were of analytical grade.

2.2. Methods

2.2.1. Preformulation studies

2.2.1.1. Drug characterization. Drug sample was characterized for its organoleptic properties and UV absorbance. Tinidazole solution (10 ug/ml) was prepared in different solvents i.e. methanol (Okunrobo, 2007), 0.1 N Hydrochloric acid, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 respectively in volumetric flask. Prepared solutions were analyzed in a wavelength range of 190 to 800 nm.

2.2.1.2. Solubility studies(Higuchi, 1963). Tinidazole was tested for its solubility in various common solvent viz. water, acetone, chloroform and methanol as method describe by Higuchi in 1963. In short, excess amount of Tinidazole was dispersed in 10 ml solvent in 25 ml volumetric flask at room temperature and kept it on biological shaker for 72 h. Filter the content through Whattman filter paper no-42 at the end and analyse the filtrate by US spectrophotometer for Tinidazole concentration.

2.2.1.3. Partition coefficient study (Jokipii et al., 1977). Aqueous drug solution (1 mg/ml) of Tinidazole was mixed with equal volume of n-octanol in 100 ml separating funnel, and was shaken vigorously for 10 min. The mixture was then allowed to stand for 24 h at room temperature. After separation, both the phases were assayed using U.V. spectrophotometer for the drug concentration in each compartment and log P value was calculated.

2.2.1.4. Drug excipients interaction study (Chauhan et al., 2009). Drug – excipient interaction study was performed to determine any physical and chemical interaction in between formulation ingredient. It was determined by taking equimolar weight of Drug and polymer, mixed them thoroughly and kept under airtight container for 30 days. Examined this mixture on 30th days by U.V. spectrophotometric method and FTIR studies for any degradation of drug and change in its physical and chemical property.

2.2.2. Radiolabelling of Tinidazole with ^{99m}Tc (Sharma et al., 2013)

Tinidazole was radilolabeled with ^{99m}Tc-pertechnetate using stannous chloride (SnCl2) as a reducing agent in dry form as per optimized protocol. ITLC (Instant thin layer chromatography) was used to ascertain the labeling efficiency. In this method, a drop of the radiolabeled Tinidazole was applied on to stationary phase (ITLC strips) and run in 100% acetone as mobile phase. The strips were dried and cut in to two equal halves and radioactivity was counted in each half by using well type gamma counter (CAPRAC-R, Capintec, USA).

Labeling efficiency was calculated using the formula:

$$\%\mathrm{LE} = \frac{\mathrm{BC}}{\mathrm{TC}} \times 100$$

Where BC stands for counts in bottom parts and TC stands for total radioactivity counts in ITLC. Stability studies were performed for optimized ^{99m}Tc-Tinidazole to evaluate any leaching of ^{99m}Tc-Tinidazole complex on storage at room temperature (saline stability) or in presence of biological fluid (serum stability).

2.2.2.1. Saline stability (Sharma et al., 2013). An in-vitro stability of radio labeling was performed by mixing 100 μl of ^{99m}Tc-Tinidazole with 900 μl of saline, allowed to vortex it for proper mixing and kept at room temperature. Small aliquots were withdrawn at different time intervals up to 24 h and radiochemical purity of ^{99m}Tc-Tinidazole was evaluated by standard ITLC method using acetone as mobile phase. The developed strips were cut in two equal halves and radioactivity in each part was measured to calculate the stability of the ^{99m}Tc-Tinidazole.

2.2.2.2. Serum stability (Sharma et al., 2013). An in-vitro serum stability study was also performed by mixing 100 μl of ^{99m}Tc-Tinidazole with 0.9 ml of Biological fluid (serum), vortex it for proper mixing and kept at 37 °C. Small aliquots were withdrawn at various time intervals up to 24 h and radiochemical purity of drug was evaluated by standard ITLC using acetone (mobile phase). The developed strips were cut in two equal halves and radioactivity in each part was measured to calculate the stability of the ^{99m}Tc-Tinidazole.