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Formulation and evaluation of a novel mucoadhesive drug delivery system to treat intestinal candidiasis in immunocompromised patients

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The limited solubility, and therefore bioavailability, of the antimycotic drug, itraconazole, used for the treatment of intestinal Candidiasis in immunocompromised individuals, has been well documented. Therapeutic regimen in these patients may include daily administration of multiple doses of various drugs. Hence, improving the residence time of therapeutic agents, would ensure a high continuous concentration in the body and help decrease the dosing frequency. In our current study, we have investigated a novel method of drug delivery, developed by utilizing the concept of mucoadhesiveness, for the sustained release of the drug, itraconazole. Mucoadhesive beads were prepared using two natural polymers, isabghula husk and alginate. The minimum inhibitory concentration of itraconazole for *Candida* was found to be 1.5milligram per millilitre. Accordingly, beads were prepared by ionic gelation method using calcium chloride as a crosslinking agent. Marked improvement in solubility of the drug was noted after entrapment. Prepared beads were subjected to various evaluations including particle size, swelling behaviour and mucoadhesivity. At pH 7.4, good mucoadhesive property was exhibited up to 7 hours. Maximum swelling of beads was observed at 4hours in phosphate buffer after which the beads showed slight erosion. Fresh cock intestinal mucosa was used to assess the sustained release of itraconazole from the drug loaded beads and the reduction in candidal cells adhering to the mucosa was verified by the viable count technique. The results of our present study indicate that mucoadhesive intestinal retentive isabghula- alginate beads could represent a promising vehicle for drug delivery and help improve therapeutic efficacy and patient compliance in the future.

Keywords: Isabghula, sodium alginate, mucoadhesion, *Candida*

Candida albicans is a yeast that constitutes the normal flora of the human gastrointestinal tract. However, disequilibrium of the microbiota and dysregulation of the immune system in immunocompromised patients such as bone marrow transplant recipients and patients with acquired immunodeficiency syndrome (AIDS), favours the growth of these opportunistic microorganisms. The incidence of gastrointestinal *Candida* infections has increased in Human immunodeficiency virus (HIV) infected patients in the last two decades (Ghannoum *et al*, 2001). Patients with HIV infection are most likely to develop candidiasis after their CD_4 counts have dropped to below 200 cells per cubic millimetre. Prophylactic antifungal agents like itraconazole can prevent the development of these infections. The management of HIV infection along with

other opportunistic diseases involves daily administration of multiple drug dosages. This is of particular concern, since the intensive therapeutic regimen followed by these patients is time consuming and cumbersome. The aim of our investigation was to explore a drug delivery system for the sustained release of itraconazole, in HIV infected patients suffering from intestinal candidiasis, utilizing the concepts of mucoadhesiveness. Our study describes the formulation and evaluation of intestinal-mucoadhesive beads of alginate containing the anti-fungal agent itraconazole, employing isabghula as a mucoadhesive material.

Isabghula or psyllium (*Plantago ovate* Forsk.) is an important ayurvedic herb belonging to the Plantaginaceae family. Its seeds and husk are used for medicinal purposes. The seeds of isabghula contain mucilage (10-12%), fatty oil and large quantities of albuminous matter which is a pharmacologically inactive glucoside (Chevallier, 1996). Psyllium seed husk (PSH) consists of a xylan backbone with (1-3) and (1-4) ß-D linkages and is highly substituted with arabinose / aldobiouronic acid residues (Prajapati *et al*, 2008). It is a partially fermented dietary fiber that increases stool weight and promotes laxation (Prynne *et al*, 1979; Spiller *et al*, 1979). Many studies have documented the cholesterol reducing and glycemic response properties of psyllium husk containing foods (Guido *et al*, 2004).Thermally and acid treated husk has also been used as a superdisintegrant in many formulations.

Formulations of mucoadhesive polymers are of current interest in the design of drug delivery systems. Current uses of mucoadhesive based preparations include ophthalmic solutions, local applications to treat diseases, protein and peptide delivery etc. (Malaekeh-Nikouei *et al*, 2008). A few previous investigations have demonstrated the mucoadhesive properties of isabghula to membranes (Nayak *et al*, 2010) due to its high mucilage content suggesting it as a suitable carrier for sustained drug release. PSH can swell and form a white, fibrous gel by absorbing water and can get attached to mucus linings by forming bonds on their surface. This helps to provide a more controlled and a sustained release profile of drug delivery than possible by conventional methods. It is hypothesized that the increased bioavailability of the drug for a longer time results due to a higher flux generated by the intimate contact between the surfaces of the mucosal layer and the pharmacoactive compound.

Alginates are natural polysaccharides obtained from brown algae and can be considered as block polymers consisting of mannuronic acid, guluronic acid, and mannuronic acid-guluronic acid. They are hemocompatible and do not accumulate in any organ of the body. They have also been used as matrix material in medicine to achieve controlled drug delivery due to their hydrogel forming properties. Although alginates also have mucoadhesive properties, its beads are usually fragile (George *et al*, 2006; Sandhu *et al*, 1981) and can be strengthened by incorporation of isabghula husk in the formulation. Itraconazole is hydrophobic in nature and its absorption rate in the gastrointestinal tract is slow due to poor dissolution. Inclusion of itraconazole in cross-linked isabghula-alginate beads would help to overcome this difficulty and improve biosorption.

In our present investigation we have tested the two main criteria important for a successful formulation- mucoadhesion to retain the antibiotic on the surface of the gastric mucosa and sustained activity of the released drug.

Materials and methods

Culture & growth conditions

Candida albicans culture obtained from a local hospital was grown overnight at 37^{o}C in Sabouraud's broth to obtain cells in budding phase. Cells were harvested,

washed in sterile saline and standardized to an O.D. of 0.1 at 530nm (Erma Inc.).

The antifungal agent, itraconazole (ITZ) used in this study was procured from Johnson & Johnson (Mumbai). Ispaghula husk was purchased from a local market (Telephone Brand, Gujarat) and sodium alginate from S.D.Fine Chemicals, Mumbai. All other chemicals used were of analytical grade.

Determination of minimum inhibitory concentration of Itraconazole against *C. albicans*

Using stock solution of itraconazole (10mg/ml) various dilutions were prepared. 0.1 ml of the 48 hour old *C.albicans* culture was added to all the tubes. Positive and negative controls were maintained. Tubes were incubated at 37^{o}C for 48 hour and the lowest concentration that did not show growth corresponded to the minimum inhibitory concentration (MIC).

Preparation of alginate-isabghula beads loaded with itraconazole

Alginate-isabghula beads containing itraconazole were prepared by employing the ionotropic gelation method using calcium chloride ($CaCl_2$) as a counter ion (Hudson *et al*, 1995). Dispersions of sodium alginate and isabghula were prepared separately in distilled water. Both dispersions were homogenized with a magnetic stirrer. The active substance, itraconazole, was added to the isabghula dispersion. Drug concentration was maintained according to the MIC of itraconazole for *Candida.* Both the dispersions were then mixed in a ratio of 1:1 and stirred at 1000 rpm for 10 minutes. The resulting dispersion was then added via a gauze needle (no. 23) into agitated $CaCl_2$ solutions (6 and 7% w/v concentration). The resultant beads were retained in the $CaCl_2$ solution for 20 minutes to complete the curing reaction and to produce spherical rigid beads. Beads were collected by decantation and washed with deionized water. Drug loaded beads were dried in a hot air oven at 40 °C for 48 hours.

Drug entrapment

50 mg of beads were accurately weighed and dispensed in 100 ml of phosphate buffer pH 7.4 and kept for 48 h at 37°C with occasional shaking. The polymer debris formed after disintegration of the beads was removed by filtering through Whatmann filter paper (No. 40). The drug content in the filtrate was determined using a UV-Vis spectrophotometer (Helios- alpha) at 274 nm.

Particle size measurement

To determine the particle size of itraconazole-loaded beads, 100 dry beads were measured using an optical microscope (Leitz Wetzlar, Germany) with an ocular micrometer.

Mucoadhesion testing by in vitro wash-off method

In order to check the mucoadhesive property of the beads, in vitro wash-off method was used. (Sharma *et al*, 2009; Prajapati *et al*, 2008). Freshly excised pieces of cock intestinal mucosa (1 cm x 1 cm) were mounted on a glass slide using cyanoacrylate glue. About 50 beads were spread out on each piece of mucosa and suspended in a 500ml vessel containing 900 ml phosphate buffer (pH 7.4) maintained at 37°C. The tissue specimen was given a regular up and down movement, to simulate a tablet disintegrator, and the adherence of beads was regularly observed. The beads that remained adhered to the mucosa were counted upto 10 hours at regular intervals.

Evaluation of swelling behaviour

Swelling behaviour was studied by measuring the percentage water uptake by the beads which is also known as swelling

index. Accurately weighed beads were placed in 50 ml of phosphate buffer (pH 7.4) and 0.1 N HCl (pH 1.2). Beads were removed from their respective swelling media after intervals of 1 hour and weighed after drying the surface water using filter paper. The water uptake was calculated as follows:

Swelling Index= $\frac{W_1 - W_2}{W_2} \times 100$

where,

W_1= Weight of beads after swelling

W_2=Weight of dry beads

Antifungal activity of the isabghula-alginate beads

To study the antifungal activity of the prepared beads, 0.1 ml of *Candida* culture (adjusted to OD 0.03) was added to several small pieces of cock intestinal mucosa (1cm x 1cm) and allowed to adhere for 24hours. These pieces were then washed with phosphate buffer (pH7.4) and treated with ITZ-loaded beads for different time intervals. Viable count of candidal cells was determined by suspending and vortexing the mucosal pieces in sterile saline, followed by plating on modified Malt extract agar containing penicillin, streptomycin and potassium tellurite. Plates were incubated at 37^{o}C for 48 hours and colonies counted. Controls were maintained and consisted of sets of intestinal tissue treated with *Candida* culture but not exposed to ITZ-loaded beads. All experiments were performed in triplicate and repeated three times. Mean values have been reported.

Results

The minimum inhibitory concentration of itraconazole for *Candida* was found to be 1.5 milligrams per millilitre by the macro-broth dilution method. Hence, this concentration was selected for incorporation in the ispaghula husk-sodium alginate beads, prepared by ionic gelation method. Beads formed were spherical, free flowing and cream in colour. The drug content entrapped was found to be 1.9 mg per 50mg beads. Their average diameter was between 0.89- 0.97millimeter, while the ideal concentration of $CaCl_2$ for formation of rigid beads was determined to be 7%.

The swelling behaviour of mucoadhesive polymeric beads is one of the major factors controlling the release of the drug from them.

Figure 1: Swelling Index of isabghula-alginate beads containing itraconazole in phosphate buffer and HCl at intervals of one hour.

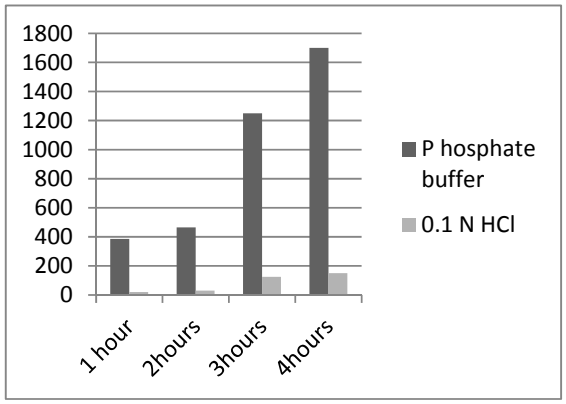

Table 1: Mucoadhesivity of isabghula-alginate beads at different time intervals.

Time (Hours)	Percentage of beads (%)
0	100
1	96±1.6
2	82±2.4
3	78±1.1
4	72±1.2
5	60±1.8
6	46±1.2
7	36±1.2
8	-
9	-
10	-

Table 2: Viable count of candidal cells adhered to mucosal surface at different time intervals

	0 hour	2hours	5hours	18 hours
Test	$1.9x\ 10^9$	$2.1x10^5$	$1.2x\ 10^3$	No growth
Control	$1.7x10^9$	$2.9x10^9$	$4.1x\ 10^7$	$1.5x\ 10^7$

In vivo, swelling of the beads would occur during their transit through the gastrointestinal tract, that is, first in the stomach and then in the intestine. For this reason, the swelling behaviour of ITZ-loaded alginate-ispaghula beads was evaluated in hydrochloric acid as well as in phosphate buffer (representing the pH of stomach and intestine respectively) at various time intervals. Maximum swelling of the capsules was noticed at 4hours in phosphate buffer, after which the beads showed a slight erosion. Figure 1 shows the increase in the swelling index (S.I.) with time. Mucoadhesivity studies were performed by assessing the degree of adherence of the beads to pieces of intestinal mucosa in the presence of phosphate buffer (pH 7.4).Our results (Table 1) showed adherence of 36% of the beads upto 7 hours following which complete disintegration of the capsules occurred. Viable count of candidal cells, determined by the surface spread technique revealed small black colonies on plates after 48 hours. A 10,000-fold decrease in the number of viable cells was evident after exposure to the drug loaded beads for 2 hours. Complete killing of all the yeast cells was noted in 18 hours. The result of viable count of yeast cells, at different time intervals, is shown in Table 2.

Discussion

In the last few decades there has been an explosion in the number of technologies available to control drug distribution and bioavailability (Bajaj *et al,* 2006; Pillai *et al,* 2011). However, there is still need for further research to develop novel drug delivery systems (NDDS) which can improve therapeutic efficacy and decrease the dosing frequency during antimicrobial therapy. In this paper, we have focused on improving the drug bioavailability and hence patient compliance in HIV-infected individuals suffering from intestinal candidiasis, by itraconazole. Itraconazole was selected as a drug of choice since resistance has emerged to fluconazole, the primary azole antifungal agent being used to treat *Candida* infections. Also other negative aspects of fluconazole, like drug toxicity and interactions, make itraconazole a far more suitable choice for prophylaxis and treatment for fungal infections of the gastrointestinal tract. Itraconazole inhibits fungal cytochrome P450 oxidase leading to impaired ergosterol metabolism. Studies have also shown it to modify the cell wall of *Candida* and provoke defective separation between mother and daughter yeast cells (Bastide *et al.* 1987). Itraconazole has relatively low bioavailability after oral administration, especially when given in capsule form on an empty stomach. In our current study we found that entraption of itraconazole in isabghula beads helped to increase its solubility, which would thus serve to increase its biosorption and residence time in the intestine.

The isabghula-alginate capsules exhibited good mucoadhesive property in the *in vitro* test. Drug release from the ITZ loaded beads was slow as adhesion to intestinal mucosa extended over an 8 hour period at pH 7.4. Mucoadhesion occurred

due to swelling of the natural polymers isabghula and alginate at an alkaline pH of the gut, followed by diffusion of the hydrophilic groups of these polymers into the mucosal layer. Strong secondary bond formation resulted in stable mucoadhesion for a long time, helping in sustained drug delivery until the beads underwent erosion. (Mohammed *et al,* 2010). Our results of mucoadhesion testing are in agreement with studies by previous investigators who have employed isabghula as a mucoadhesive polymer for delivery of glucose lowering agents, metformin hydrochloride and gliclazide (Sharma *et al*, 2008; Prajapati *et al*, 2008). To our knowledge, this is the first study to explore the use of isabghula as a mucoadhesive agent to enhance the delivery of an antimicrobial agent to the intestine for treating a mycotic infection. Theresults of viable count of cells show itraconazole as a very effective drug to control candidiasis. Conventionally, itraconazole dosages are administered daily, after a 12 hour interval. Our results show mucoadhesive beads could eliminate all viable cells even up to 18 hours, thus indicating that this mode of drug delivery could greatly help in decreasing the dosing frequency in a patient. The 100 fold decrease in number of viable candidal cells in the control after 18 hours, could probably be due to the absence of sugars which could have served as a nutrient source on the mucosal tissue. In vivo, the dietary intake of sugars and carbohydrates by an individual would ensure that such a decrease in the population of viable cells would be unlikely to occur without the administration of an antimycotic agent.

Conclusion

Today the field of developing novel techniques of drug delivery represents a major research and development focus area, in view of the manifold therapeutic benefits which can be availed of by patients. The development of intestinal controlled release formulations continues to be one of the greatest challenges in the pharmaceutical industry due to the vagaries of the gastrointestinal environment.

Drug loaded beads, prepared using alginate and psyllium husk, both natural polymers, were found to be efficient with respect to swelling ratio, mucoadhesion and antifungal activity. Hence, our investigation indicates the potential benefit of using isabghula as a mucoadhesive agent for intestinal controlled release of antimycotic itraconazole for the treatment of intestinal candidiasis in HIV infected patients. Further in vivo tests and clinical trials would validate the usefulness of this approach and lead to the introduction of a novel mode of therapeutic delivery.

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