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Research Paper

Effect of co-administration of probiotics with polysaccharide based colon targeted delivery systems to optimize site specific drug release

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ABSTRACT

Significant clinical success of colon targeted dosage forms has been limited by their inappropriate release profile at the target site. Their failure to release the drug completely in the colon may be attributed to changes in the colonic milieu because of pathological state, drug effect and psychological stress accompanying the diseased state or, a combination of these. Alteration in normal colonic pH and bacterial picture leads to incomplete release of drug from the designed delivery system. We report the effectiveness of a targeted delivery system wherein the constant replenishment of the colonic microbiota is achieved by concomitant administration of probiotics along with the polysaccharide based drug delivery system. Guar gum coated spheroids of sulfasalazine were prepared. In the dissolution studies, these spheroids showed markedly higher release in the simulated colonic fluid. *In vivo* experiments conducted in rats clearly demonstrated the therapeutic advantage of co-administration of probiotics with guar gum coated spheroids. Our results suggest that concomitant use of probiotics along with the polysaccharide based delivery systems can be a simple strategy to achieve satisfactory colon targeting of drugs.

1. Introduction

Earlier considered to be a disease of developed and industrialized countries, ulcerative colitis (UC) has seen a rampant rise in developing countries [1]. Past few decades have witnessed a sharp rise in the cases of Inflammatory Bowel Disease (IBD) in Asia [2]. Incidence rate of UC is reported to vary from 0.5 to 31.5 per 10^5 persons worldwide [3]. In Asia, however, it is reported as 5.3–53.6 per 10^5 [4,5], as compared to 37.5–238 per 10^5 persons in North America [6].

UC is a pathological condition characterized by chronic inflammation of large bowel. Patients suffer from bloody diarrhoea and generally the stool culture does not show the presence of bacteria or parasites. A number of factors contribute towards the etiopathogenesis of the disease. These include breakdown in patient's immune system [7], abnormal mucosal immune response against colonic microflora [8], environmental factors [9], genetic factors [10,11] and oxidative damage [12].

Human gut is host to a plethora of microorganisms, majority comprising of bacteria, though some fungi and protozoa also exist [13,14]. Commensal bacteria play an important role in maintaining the health of an individual. They prevent the predominance of pathogenic bacteria as they compete with them for space and food. They also produce certain molecules which have antibacterial properties and prevent the colonization by pathogenic bacteria [15].

UC is always accompanied by an imbalance between the beneficial and harmful bacteria in the colon. There is an overall decrease in *Bacteroides*, particularly *Bacteroides vulgates* [16], *Eubacterium*, *Lactobacillus* and *Bifidobacteria* and an increase in *E. coli* and *Clostridia* in IBD patients [17]. At some point, the immune tolerance to colonic microflora is broken and the host activates immune reaction against the colonic bacteria [18]. This causes inflammation of mucosa which is a characteristic feature of UC [13].

Conventional treatment of UC is aimed at reducing or eliminating the inflammation of colonic mucosa, as well as, combating other symptoms of the disease. Traditionally, aminosalicylates have played a central role in treatment of the disease [19]. Other alternatives are corticosteroids, immunomodulators such as azathioprine (AZA), 6-mercaptopurine (6MP), infliximab and cyclosporine [19,20]. Drugs such as steroids, AZA, 6 MP, and

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cyclosporine have severe side effects on long term usage. Sulfasalazine (SFZ) is the cornerstone drug in treatment of UC, which has been found to be superior to corticosteroids in reducing the number of relapses in patients on maintenance therapy. Currently SFZ is considered as the drug of choice for the treatment of UC. Chemically, it consists of 5-aminosalicylic acid attached by diazo bond to sulfapyridine moiety [21]. Diazo bond is broken by the enzymes secreted by colonic bacteria [22], resulting in the liberation of 5-aminosalicylic acid and sulfapyridine. 5-Aminosalicylic acid is the pharmacologically active moiety that is responsible for anti-inflammatory properties of the drug, whereas sulfapyridine is responsible for most of the side-effects; main side effects being Heinz body anaemia and agranulocytosis. Other frequently occurring side-effects are fever, arthralgia and rashes [19]. As the absorption of SFZ from colon is poor [23], its delivery to colon is expected to reduce its side effects. Moreover, any delivery system that helps in delivering the drug at the target site (i.e. colon) and thus helps in reducing the exposure of non-target sites of the drug would be worth exploring.

The most widely used approaches to formulate colon-specific drug delivery systems include timed release systems, pressure controlled systems, osmotically controlled systems, pH sensitive polymer coating, prodrugs and colonic microflora activated delivery systems [24–28]. Use of the mentioned approaches is, however, limited by a number of inherent disadvantages [29–32]. The best alternative approach for colon specific drug delivery is the use of carriers that are digested by the action of colonic microflora. These carbohydrate carriers include pectin and its salts, chondroitin sulphate, amylose, inulin HP, guar gum and many more [33–36]. An abrupt increase of the bacterial population and associated enzymatic activities in the ascending colon represent an exclusive and non-continuous event, independent of GI transit time and pH [37–39]. In our recent report, we have extensively discussed about various microflora activated polysaccharide based colon targeted drug delivery systems inclusive of their advantages and limitations [40].

Sulfasalazine has been reported to reduce the number of colonic anaerobic bacteria, which adversely affects the gastrointestinal physiology [41]. This, in turn, increases the chances of opportunistic infections which can further aggravate the situation. The effectiveness of probiotics has already been proven in the restoration of normal microfloral picture in colon. A number of studies point towards positive effect of prebiotics and probiotics on the symptoms of IBD [42]. These effects are probably the result of change in composition of microflora caused by probiotics as well as modulation of gut immune system [43]. Thus co-administration of probiotics with polysaccharide based delivery system is aimed at providing these additional benefits besides facilitating their targeted release in colon.

In the present study, sulfasalazine spheroids were developed which were coated with guar gum. Another formulation of spheroids was prepared where coating was done with a mixture of guar gum and Eudragit®. The developed spheroids were subjected to physical characterization, *in vitro* and *in vivo* evaluation studies. The effect of co-administration of probiotics on the therapeutic efficacy of prepared sulfasalazine spheroids was studied in the rat model of UC.

2. Materials and methods

2.1. Materials

Sulfasalazine (SFZ) was purchased from Swapnroop Drugs & Pharmaceuticals, India. Probiotic (BIOMIX-I) was a gift from Unique Biotech, India. Guar gum, sodium hydroxide pellets, hydrochloric acid, and isopropyl alcohol were from Loba chemie, India. Microcrystalline cellulose PH 101 was procured from Jackson laboratories, India. Talc was purchased from Qualikems Fine, India. Hexadecyltrimethylammonium bromide was purchased from Sigma–Aldrich, India. Eudragit® S100 was a gift sample from Evonik Pharma, India. Millipore water was used throughout the study. Dissolution apparatus used was LAB INDIA DS 8000, India.

2.2. Methods

2.2.1. Preparation of sulfasalazine (SFZ) spheroids

For a batch of 30 g, spheroids containing 40% Sulfasalazine (12 g), 40% guar gum (12 g), 18% microcrystalline cellulose PH 101 (5.4 g) and 2% talc (0.6 g) by weight ratios were prepared by extrusion and spheronization [44–46]. The size range was adjusted between 1 and 1.2 mm. The blend of drug and excipients was wetted by a mixture of isopropyl alcohol and water. This wet mass was subjected to extrusion (RRE/EXT-05/037, R.R. Enterprises, India). The extruded mass was then subjected to spheronization (Spheronizer REE/SPH-150/010) which was carried out at 2000 rpm for 20 min. The obtained spheroids were dried in tray dryer (CADMACH drying oven, India) at 40 °C for 1 h.

2.2.2. Coating of prepared spheroids

The coating solution of Eudragit® S100 was prepared by dispersing 5 g of Eudragit® S100, 2.5 g PEG 6000 (plasticizer) and 2.5 g of talc in 100 ml of acetone–isopropyl alcohol mixture (50:50% v/v). The prepared mixture was stirred for 1 h on a magnetic stirrer to make a homogeneous solution.

The coating solution of guar gum was prepared by dispersing 5 g of guar gum in 100 ml of water–isopropyl alcohol mixture (90:10% v/v). The prepared mixture was stirred for 2 h on a magnetic stirrer to make a homogeneous slurry.

To achieve site specific delivery of prepared spheroids of sulfasalazine, different coating strategies were used. Ten different batches (B-2 to B-10) were subjected to coating using different ratios of core to coating solution. The coating was done using either Eudragit® S100 or guar gum. A binary coating of guar gum followed by Eudragit® was also tried. Composition of various coating solutions used is given in Table 1. For the preparation of different coated batches of sulfasalazine spheroids, 20 g of uncoated spheroids was taken in fluidized bed coater (Mini Glatt, Pam Glatt Pharma Technologies Pvt. Ltd., India). The coating solution was preheated to 60 °C prior to the coating process. Inlet temperature was maintained between 45 and 60 °C, and spraying rate was 0.4–0.6 ml/min at atomizing air pressure of 0.2–0.3 Bar. Based on the nature of coating polymers used, the coated spheroids were kept for 1–2 h at 40–60 °C. The film thickness was calculated as the percentage of the weight gained relative to the weight of coated pellets [46].

Table 1
Different batches of spheroids of sulfasalazine with different percentage of coating polymers.

S. no.	Formulation batches	Coating polymer percentage
1	B-1	Uncoated
2	B-2	5% w/w Guar Gum
3	B-3	10% w/w Guar Gum
4	B-4	15% w/w Guar Gum
5	B-5	20% w/w Guar Gum
6	B-6	5% w/w Guar Gum + 5% w/w Eudragit® S100
7	B-7	5% w/w Guar Gum + 10% w/w Eudragit® S100
8	B-8	5% w/w Guar Gum + 15% w/w Eudragit® S100
9	B-9	10% w/w Guar Gum + 10% w/w Eudragit® S100
10	B-10	15% w/w Guar Gum + 15% w/w Eudragit® S100

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