

PROBIOTIC ASSISTED COLON TARGETED DRUG DELIVERY SYSTEM: RESEARCH SCOPE

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ABSTRACT

Manipulation of the gut micro flora is considered a potential instrument to modulate colon disorders. In this view, probiotics, defined as "live microbial food ingredients that are beneficial to health" are considered to have the potential to reduce the colon disorders by stimulating the immune system, decreasing the incidence of infections, regulating gut inflammation, and binding toxic evidence exists showing that the administration of probiotics, alone or in combination with fermentable carbohydrates as prebiotic, is able to modify colonic microflora and positively affects the processes leading to modification of the intestinal environment and favouring the development of preneoplastic or neoplastic lesions. Targeted drug delivery to the colon would therefore ensure direct treatment at the disease site, lower dosing and fewer systemic side effects. In addition to local therapy, the colon can also be utilized as a portal for the entry of drugs into the systemic circulation.

Keywords: Colon-specific drug delivery, Polysaccharides, Probiotics, Micro flora etc.

INTRODUCTION

Drug delivery systems to the colon are being extensively investigated in order to treat local colonic diseases like irritable bowel syndrome, crohn's disease and ulcerative colitis^{1,2}.

The most promising colon-specific drug delivery systems are those based on the enzymatic action of colonic bacteria on polysaccharides. The microflora of colon is in the range of 10^{11} - 10^{12} CFU ml⁻¹ consisting mainly of anaerobic bacteria, e.g. *Bacteroides*, *Bifidobacteria*, *Eubacteria*, *Clostridia*, *Enterococci*, *Enterobacteria* and *Ruminococcus* etc. This vast microflora fulfills its energy needs by fermenting various types of substrates that have been left undigested in the small intestine, e.g. di- and tri-saccharides, polysaccharides etc. For the fermentation of these polysaccharides, the microflora of GIT produces a vast number of enzymes like β -glucuronidase, β -xylosidase, α -arabinosidase, β -galactosidase, nitroreductase, azareducatase, deaminase and urea dehydroxylase. Because of the presence of these enzymes only in the colon, the use of biodegradable polymers for colon-targeted drug delivery seems to be a more site-specific approach when compared to other approaches^{3,4}. The ability of colon microflora to degrade polysaccharides such as pectin, guar gum, chitosan, etc. forms the basis of formulation development for colon-specific drug delivery^{5,6}. The colonic microflora must be present in sufficient number to digest the carrier (like guar gum) to ensure the release of the drug at colon. However, intersubject variation of colonic microflora, sterilization of colonic microflora by antibiotics (commonly prescribed to treat colonic disease) and slow enzymatic degradation are some limitations of this approach for colon-specific drug delivery. The limitations can be resolved by incorporating bacteria in spore form (probiotics) in formulation to assure successful and efficient drug release selectively to colon. Probiotic supplements (*Bifidobacteria spp* and *Lactobacilli*) are known to improve resistance to gut infections by inhibiting the growth of harmful bacteria, to reduce cholesterol levels, improve the immune response and produce vitamins. Various probiotic-containing preparations like Sporlac (*Lactobacillus*) and Prowell (*Bifidobacterium*) are available in the market in spore form. Protective effect of probiotics (lactic acid producing bacteria) in colon carcinogenesis has also been demonstrated in clinical trials. Incorporation of probiotics would provide the health benefits on one hand, while ensure drug release at colon in any condition on the other⁷⁻¹⁰.

Polysaccharides

Polysaccharides are polymers of monosaccharides (sugars). They are found in abundance, have wide availability, are inexpensive and available in a variety of structures with a variety of properties. They can be easily modified chemically and biochemically and are highly stable, safe, nontoxic, hydrophilic and gel forming and in addition

biodegradable, which suggests their use in targeted drug delivery systems. Problem encountered with the use of polysaccharides is their high water solubility. An ideal approach is to modify the solubility while still retaining their biodegradability. Large number of polysaccharides has already been tried for their potential as colon-specific drug carrier systems, such as chitosan, pectin, chondroitin sulphate, cyclodextrins, dextrans, guar gum, inulin, pectin, locust bean gum and amylose¹¹.

Micro flora Activated System

The basic principle involved in this method is degradation of polymers coated on the drug delivery system by microflora present in colon and there by release of drug load in colonic region because the bioenvironment inside the human GIT is characterized by presence of complex microflora, especially the colon is rich in microorganisms. In this method drugs and/or dosage forms are coated with the biodegradable polymers i.e., the polymers degrade due to influence of colonic microorganisms. When the dosage form passes through the GIT, it remain intact in the stomach and small intestine where very little microbial degradable activity is present which is insufficient for cleavage of the polymer coating^{12,13}.

Drug candidates for colon delivery

Theoretically, any drug can be a candidate for colon targeted drug delivery. However only those drugs, which show poor bioavailability from the stomach or intestine and peptide drugs, are the most suitable for colonic targeting. The ideal drug candidates for colonic drug delivery include agents that are useful for disorders such as IBD, ulcerative colitis, amoebiasis and colon cancer^{14,15}.

Fibre fermentation by gut bacteria

Foods rich in dietary fibre include vegetables, fruit, cereal grains and legumes. Dietary fibres display different degrees of solubility. Some such as pectins, hemicellulose, guar gum and inulin are readily soluble in water²¹. This leads to the formation of gels in the gastrointestinal tract. This aids their fermentability by the gut microflora by virtue of an increased surface area available for enzymatic attack. The relative fermentability of different fibres is dependent on a number of physicochemical properties. Fibre particle size and degree of solubility have a considerable effect on the susceptibility of fibres to bacterial fermentation in that they govern the surface area exposed to bacterial degradation. The fermentation of dietary fibre in the colon has a number of attributes. The main product of polysaccharide fermentation in the colon is bacterial biomass, which not only increases stool bulk but gives rise to increased numbers or metabolic activity of the main saccharolytic bacterial species.

Table 1: Drug Candidates

Criteria	Pharmacological Class	Drug Candidate	
		Non-Peptide Drugs	Peptide Drugs
Drug used for local effects in colon against GIT diseases	Anti-inflammatory drug	Oxyprorenolol, Metoprolol, Nifedipine, Diclofenac sodium	Amylin, Antisense oligonucleotide, Calcitonin
Drug poorly absorbed from upper GIT	Antihypertensive and antianginal drugs	Ibuprofen, Isosorbides, Theophylline	Cyclosporine A, Desmopressin
Drug for colon cancer	Antineoplastic drugs	Pseudoephedrine	Epoetin, Glucagon
Drug that degrade in stomach and small intestine	Peptides and proteins	Bromophenaramine, 5-Fluorouracil, Doxorubicin	Gonadoreline, Insulin, Interferons
Drugs that undergo extensive firstpass Metabolism	Nitroglycerin and Corticosteroids.	Nimustine, Bleomycin, Nicotine, Dexamethasone	Molgramoatim, Protirelin, Sermorelin, Saloatonin
Drugs for targeting	Antiarthritic and antiasthmatic drugs	Prednisolone, Hydrocortisone, 5-Amino-salicylic acid (5-ASA)	Somatropin, Urotoilitin, Vasopressin

Table 2: Successful probiotic bacteria and their reported effects¹⁶⁻²⁰

Sr.no	Strain	Reported effects in clinical studies
1	<i>Lactobacillus acidophilus</i> LA1	Immune enhancer, adjuvant, adherent to human intestinal cells, balances intestinal microflora
2	<i>Lactobacillus acidophilus</i> NCFB 1748-	Lowering faecal enzyme activity, decreased faecal mutagenicity, prevention of radiotherapy related diarrhoea, treatment of constipation
3	<i>Lactobacillus</i> GG (ATCC 53013)	Prevention of antibiotic associated diarrhoea, treatment and prevention of rotavirus diarrhoea, treatment of relapsing <i>Clostridium difficile</i> diarrhoea, prevention of acute diarrhoea, Crohn's disease, antagonistic against carcinogenic bacteria, vaccine adjuvant
4	<i>Lactobacillus casei</i> Shirota	Prevention of intestinal disturbances, treatment of rotavirus diarrhoea, balancing intestinal bacteria, lowering faecal enzyme activities, positive effects in the treatment of superficial bladder cancer, immune enhancer in early colon cancer, immune enhancement
5	<i>Streptococcus thermophilus</i>	No effect on rotavirus diarrhoea, no immune enhancing effect during rotavirus diarrhoea, no effect on faecal enzymes
6	<i>Bifidobacterium bifidum</i>	Treatment of rotavirus diarrhoea, balancing intestinal microflora, treatment of viral diarrhoea
7	<i>Lactobacillus gasseri</i> (ADH -)	Faecal enzyme reduction, survival in the intestinal tract
8	<i>Lactobacillus reuteri</i>	Colonising the intestinal tract, mainly animal studies so far, possibly an emerging human probiotic

Increased stool bulk contributes towards reduced colonic transit times which is seen as beneficial not only for the relief and prevention of constipation, but in reducing the impact of detrimental microflora associated characteristics such as toxic nitrogenous compounds, H₂S, and the production of carcinogenic or genotoxic compounds. Dietary fibre contributes towards stool bulking through increased bacterial biomass and more directly through the sheer volume of fibre compounds such as the lignins and residual cellulose with escape bacterial fermentation. Bacterial fermentation also results in a lowering of colonic pH. Lower pH values impede the growth of certain pathogenic bacterial species while encouraging the growth of the bifidobacteria and lactic acid microflora. A low colonic pH may also aid in the excretion of carcinogens, which bind to dietary fibre in the colon²². SCFA, being the main acidic products of bacterial fermentation, contribute towards a low colonic pH. The organic moieties of SCFA have also been shown to have a direct inhibitory activity towards important gastrointestinal pathogens. SCFA, as mentioned above, are also metabolised by host tissues. Surprisingly few data exist on the ability of dietary fibre to act as prebiotics.

In particular, a confirmation on the selectivity of fermentation is not apparent, especially using high fidelity molecular based procedures for determining flora changes as a response to the fermentation. As such, their capacity to stimulate probiotics in the gut is unproven. More research is required, however promise does exist with derivatives of common fibres. For example, one of the best characterised fibres is pectin which is not selectively metabolised by the gut flora. Pectic derived oligosaccharides do Prebiotic concept²⁷ however show good promise²³. This effect may be explained by the observation that probiotics like the bifidobacteria prefer to utilise carbohydrates of oligosaccharide size rather than higher molecular weight polymers. The latter do not generally have the selective metabolism required of efficacious prebiotics^{24, 25}.

Formulation and development

1. Preformulation studies
2. Preparation of matrix tablet by wet granulation technique

3. Optimizing the formulation by variation in the different formulation Variables.
4. Evaluation
 - a. Thickness
 - b. Hardness
 - c. Weight variation
 - d. Friability
 - e. Drug content
5. In-vitro release study
 - a. with RCC
 - b. without RCC

Preparation of matrix tablet by wet granulation technique

Matrix tablets were prepared by wet granulation method. Lactose was used as diluent and the mixture of talc and magnesium stearate at 2:1 ratio was used as lubricant. The powders were blended and granulated with water. The wet mass obtained was then passed through sieve no. 14 and wet granules were dried at 50°C for 2 h. The dried granules were passed through a sieve no. 16 and were lubricated with a mixture of talc and magnesium stearate (2:1). The lubricated granules were compressed at compression force 5000-6000 kg using 12-mm-round concave-convex punch on eight-station rotary tablet machine.

Formulation of matrix tablet of Polysaccharide containing probiotics

Matrix tablets were prepared using any polysaccharide by the wet granulation method using water as granulating agent. Sporlac (containing *Lactobacillus sporogens*) and Prowell (containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium infantis* spores) were used as probiotics. The method of preparation of tablets was same as described above with few modifications. Probiotics were added at two stages; half quantity was added before granulation and half quantity was added after granulation and the wet granules were dried at 40°C for 2 hr. Granules of each composition after lubrication was compressed and tested for their hardness, drug content, and drug release characteristics with the required number of tablets for each test. The hardness of the matrix tablets was determined using a Monsanto hardness tester (M/s Campbell Electronics, Mumbai, India).

In-Vitro Dissolution

In vitro dissolution studies for all matrix tablet formulations were performed by using USP dissolution test apparatus (Apparatus 1, Basket type, 37°C) at 100 rpm for 2 hr in 0.1 N HCl (900 ml). Then the dissolution medium was replaced with pH 7.4 phosphate buffer (900 ml) and tested for 3 hr as the average transit time of small intestine is 3 hr. After 5 hr, the dissolution medium was replaced with pH 6.8 phosphate buffer and tested for next 19 hr. At different time intervals a 10 ml of the sample was taken and analyzed for drug content by double beam UV-visible spectrophotometer. A 10 ml fresh dissolution medium was added to make the volume after each sample withdrawal²⁶⁻²⁹.

In vitro dissolution studies in presence of 4%w/v rat caecal content

To assess the susceptibility of guar gum being acted upon by the colonic bacteria, drug release studies were carried out in the presence of rat caecal content as described by Krishanaiya. In order to induce enzymes specifically acting on guar gum in the caecum, male Albino rats weighing 150-200 g and maintained on normal diet were intubated with teflon tubing and 1 ml of 2% wt/vol dispersion of guar gum in water was administered directly into the stomach. The tubing was removed and this treatment was continued for 3 days in one set of rats and for 7 days in another set of rats. Thirty minutes before the commencement of drug release studies, four rats were killed by spinal traction. The abdomen was opened, the caecai were isolated, ligated at both ends, cut loose and immediately

transferred into pH 6.8 PBS, previously bubbled with carbon dioxide gas (CO₂). The caecal bags were opened; their contents were individually weighed, pooled and then suspended in PBS to give a final caecal dilution of 4% wt/vol. As the caecum is naturally anaerobic, all these operations were carried out under CO₂. The drug release studies were carried out in USP dissolution test apparatus (Apparatus 1, 100 rpm, 37°C) with slight modification. A beaker (capacity 150 ml) containing 100 ml of dissolution medium was immersed in the water contained in the 1000 ml vessel, which in turn, was the water bath of the apparatus. The swollen formulations after completing the dissolution study in 0.1 M HCl (2 hr) and pH-7.4 phosphate buffer (3 hr) were placed in the baskets of the apparatus and immersed in the dissolution medium containing rat caecal content medium. The drug release studies were carried out up to 24 hr and 1 ml samples were withdrawn at specified time intervals without a pre-filter and replaced with 1 ml of fresh Sorenson's phosphate buffer bubbled with CO₂. The volume was made up to 10 ml with Sorenson's phosphate buffer and centrifuged. The supernatant was filtered and filtrate was analyzed for drug content



by double beam UV-Visible spectrophotometer^{30, 31}.

Fig 1: In-vitro drug release study in presence of RCC by USP dissolution test apparatus 1.

CONCLUSIONS

The formulation containing probiotics ensures the major portion of drug (more than 70%) to be released at colon even in absence of colonic microflora. However, *in vivo* studies are needed to confirm the results obtained in *in vitro* studies. The Polysaccharide based tablet formulation developed with probiotic holds tremendous potential to deliver a variety of drugs in colon diseases specifically at colon and ensures maximum drug concentration at colon even in case of disturbed GIT microflora on one hand while reduce the dose and frequency of the drug and consequently lowers drug-associated side effects on other.

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