An *in Vitro* and *in Vivo* Investigation into the Suitability of Bacterially Triggered Delivery System for Colon Targeting

Chellan Vijaya RAGHAVAN,* Chithambaram MUTHULINGAM, Joseph Amaladoss Josephine Leno JENITA, and Thengungal Kochupapy RAVI

College of Pharmacy, Department of Pharmaceutics, Sri Ramakrishna Institute of Paramedical Sciences, Sri Ramakrishna Hospital Campus; Coimbatore-641044, Tamilnadu, India. Received December 10, 2001; accepted March 29, 2002

The colon specific drug delivery systems based on polysaccharides; locust bean gum and chitosan in the ratio of 2 : 3, 3 : 2 and 4 : 1 were evaluated using *in vitro* and *in vivo* methods. The *in vitro* studies in pH 6.8 phosphate buffer containing 2% w/v rat caecal contents showed that the cumulative percentage release of mesalazine after 26 h were 31.25±0.56, 46.25±0.96, 97.5±0.26 (mean±S.D.), respectively. The *in vivo* studies conducted in nine healthy male human volunteers for the various formulations revealed that, the drug release was initiated only after 5 h (*i.e.*) transit time of small intestine and the bioavailability ($AUC_{0\to t}$) of the drug was found to be 85.24±0.10, 196.08±0.12, 498.62±0.10 μg h/ml 26 (mean±S.D.), respectively. These studies on the polysaccharides demonstrated that the combination of locust bean gum and chitosan as a coating material proved capable of protecting the core tablet containing mesalazine during the condition mimicking mouth to colon transit. In particular, the formulation containing locust bean gum and chitosan in the ratio of 4 : 1 held a better dissolution profile, higher bioavailability and hence a potential carrier for drug targeting to colon.

Key words locust bean gum; chitosan; colon targeting; *in vitro* study; *in vivo* study

The delivery of drugs to colon for the local effect is valuable in the variety of condition like inflammatory bowel diseases (*e.g.* ulcerative colitis and chron's disease), infectious disease and colon cancer. The colonic delivery is also useful in the systemic absorption of drugs like nifedipine, theophylline, isosorbide, *etc*. Further it is found to be a promising site for systemic absorption of peptide and protein because of less hostile environment prevailing in the colon in comparison with stomach and small intestine.[1] Additionally, the colon acts as a highly responsible site to enhance the absorption of poorly absorbable drugs.[2,3]

A colonic drug delivery system is required to protect the drug during its transit through the gastrointestinal tract and to allow its release in the colon. A number of oral systems with variety of approaches have been designed for the drug release into the colon which include a) taking advantages of the apparent consistency of small intestine transit time, b) the utilization of the pH changes within the G.I. tract and c) the exploitation of bacterial enzyme localized in the colonic region of G.I. tract. Due to the poor site specificity of pH dependent systems because of large variation in the pH of the gastrointestinal tract[4] and also the poor site specificity of the timed release dosage form because of large variation in gastric emptying time[5] made the exploitation of the bacterial enzyme localized in the G.I. tract as one of the better approach for colon targeting. Several studies were undergone on the basis of the activity of colonic bacteria on polysaccharide based carrier system. The different polysaccharides that are used under evaluation as carriers for colonic drug delivery includes pectin and its salts,[6,7] chondroitin sulphate,[8] amylase,[9] inulin HP[10] guargum.[11] This formed the basis of investigating the usefulness of the polysaccharides; locust bean gum and chitosan as a carrier for drug targeting to the colon.

The locust bean gum is a neutral polysaccharides having a molecular weight of 310000 derived from the endosperm of the seed of the ceratonia siliqua linne (Fam: leguminosae). The locust bean contains about 88% D-galacto-D-mannoglycan, 4% of pentan, 6% of protein, 1% of cellulose and 1% of ash. Chitosan is a non-acetylated or partially acetylated chitin derivative. Crustacean shells are the usual raw material for chitin. Chitosan is (1—4) 2 amino-2-deoxy β-D-glucan. It is tough, bio-degradable and non-toxic. In pharmaceutical field, locust bean gum is used as an excipient for tablets and thickener for tooth paste while the chitosan is used as an excellent direct compression aid and as a vehicle to enhance the dissolution of poorly absorbable drug.

In the present study, the locust bean gum and chitosan in the ratio of 2 : 3, 3 : 2 and 4 : 1 were applied over the core tablet in the form of compression-coat and evaluated as a carrier for colon specific drug delivery. The *in vitro* drug release studies were carried out in the simulated gastrointestinal fluids in the presence and absence of rat caecal content. The *in vivo* studies were carried out in the healthy human volunteers for their *in vivo* behavior.

Experimental

Materials Locust bean gum was obtained from Fluka Biochemica, Switzerland. Chitosan was obtained from Central Institute of Fisheries Technology, Cochin, India. Mesalazine (5-amino salicylic acid) was obtained from Sun Pharmaceuticals Ltd., India. Microcrystalline cellulose and magnesium stearate were obtained from Loba Chemie Pvt. Ltd., India. Starch was obtained from E-Merck (India) Pvt. Ltd., India. Talc and sodium lauryl sulphate were obtained from S.D. Fine Chem. Ltd., India.

Preparation of Core Tablets Each core tablet (average weight 80 mg) for *in vitro* and *in vivo* studies consists of mesalazine (40 mg), microcrystalline cellulose (29 mg), dried starch (5 mg), sodium lauryl sulphate (4 mg), talc (1.5 mg) and magnesium stearate (0.5 mg). Starch and sodium lauryl sulphate were added to obtain fast disintegration tablets (disintegration time $<$1 min) of mesalazine.

The materials were weighed, mixed and passed through a mesh (250 μm) to ensure complete mixing. The tablets were prepared by compressing thoroughly the mixed materials using 6 mm round, flat and plain punches on a single station tablet machine (Cadmach, India). The thickness of the core tablet was 2 mm. Their crushing strength were 3 kg/cm^2.

Preparation of Compression-Coated Tablets The formulated core tablets were compression-coated with different quantities of coating material such as locust bean gum and chitosan taken in the ratio of 2 : 3, 3 : 2 and 4 : 1. The microcrystalline cellulose is added in the formulation as a direct compression aid, since the locust bean gum and chitosan alone do not produce sufficient hardness.

* To whom correspondence should be addressed. e-mail: c_vijayan_2k@yahoo.com

The compression coating was provided by placing a half the quantity of coating material in the die cavity, then the core tablet was carefully positioned in the centre of the die cavity and was filled with the other half of the coating material. The coating material was compressed around the core at an applied force of 5000 kg using 9 mm round, flat and plain punches. The crushing strength of the compression-coated tablet was 5 kg/cm^2.

***In Vitro* Drug Release Studies** The compression-coated mesalazine tablets were evaluated for their integrity in the physiological environment of stomach and the small intestine under condition mimicking mouth to colon transit. These studies were carried out using a USP XXII/XXIII dissolution rate test apparatus (Apparatus 1, 100 rpm, 37 °C). The tablets were tested for drug release for 2 h in 0.1 N HCl (900 ml) as the average gastric emptying time is about 2 h. Then, the dissolution medium was replaced with pH 7.4 phosphate buffer (900 ml) and tested for 3 h as the average small intestine transit time is about 3 h. At the end of time periods, the samples from both each of 1 ml were taken separately, suitably diluted and analysed for mesalazine content using spectro-fluorimeter (Jasco FP-750).

The susceptibility of the locust bean gum and chitosan coats to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in 100 ml of pH 6.8 phosphate buffered saline (PBS) containing 4% w/v rat caecal contents. The caecal contents were obtained from male albino rats (obtained from Pasteur Institute, Coonoor, Nilgiris, India) after pretreatment for 7 d with locust bean gum and chitosan dispersion which provided the best condition for the *in vitro* evaluation of locust bean gum and chitosan. Thirty minutes before the commencement of drug release studies, five rats were killed by spinal traction. Their abdomen were opened, the caecum were isolated, ligated at both ends, dissected and immediately transferred into pH 6.8 PBS which is previously bubbled with CO_2. The caecal bags were opened, their contents were individually weighed, pooled and then suspended in PBS to give a final dilution of 4% w/v. As the caecum is naturally anaerobic, all the operations were carried out under CO_2.

The studies simulating the drug release in colon were carried out in USP XXII/XXIII dissolution rate test apparatus (Apparatus 1, 100 rpm, 37 °C) with slight modification.[11] A beaker (capacity 150 ml, internal diameter 55 mm) containing 100 ml of dissolution medium immersed in water containing 1000 ml vessel, which is in turn placed in the water bath of dissolution apparatus. The coated tablets were placed in the basket containing pH 6.8 phosphate buffered saline along with the rat caecal contents. The experiments were carried out with the continuous CO_2 supply into the beaker to simulate anaerobic environments of caecum. The drug release studies were carried out for 21 h (as usual colonic transit time is 20—30 h) and 1 ml of samples were taken at different time intervals. The volume was made up to 10 ml with PBS, centrifuged and the supernatant was filtered through a bacteria proof filter and this filtrate was analysed for mesalazine spectro-fluorimetrically.[13] The above study was carried out on all the mesalazine tablets coated with different coat composition (F1, F2, F3) and also without rat caecal content in pH 6.8 PBS (control).

***In Vivo* Studies** For the *in vivo* studies of the usefulness of locust bean gum and chitosan in the colon drug delivery, nine male healthy human volunteers of 20—23 years of age and 55—70 kg weight were selected. They were non-alcoholics, non-smokers and were not on any drugs. The purpose of the study was fully explained and each volunteer had given his written consent and been approved by the ethical committee of the institution. Their liver and kidney function were assessed to be normal by clinical and standard biochemical investigation.

A 9×3 complete cross over design was carried out in nine healthy male volunteers. After overnight fasting, the three different coated formulations (F1, F2, F3) of mesalazine were given to the volunteers along with 200 ml of water. The food was withheld for the period of 2 h. The blood samples were collected at 0, 3, 5,…31 h. The plasma was separated, the drug was extracted[12] and analyzed spectro-fluorimetrically.[13]

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

Table 1. Composition of the Compression-Coat of Mesalazine Core Tablet

Formulation	Coat weight (mg)	Composition				
		LBG	Chitosan	MCC	Mgs	Talc
F1	600	220	330	45	2	3
F2	600	330	220	45	2	3
F3	600	440	110	45	2	3

LBG: locust bean gum; MCC: microcrystalline cellulose; Mgs: magnesium stearate.

Results

***In Vitro* Drug Release Studies** The percentage of drug released at different time periods from the mesalazine tablet compression-coated with coat formulation F1, F2 and F3 in 0.1 N HCl (2 h), pH 7.4 phosphate buffer (3 h) and pH 6.8

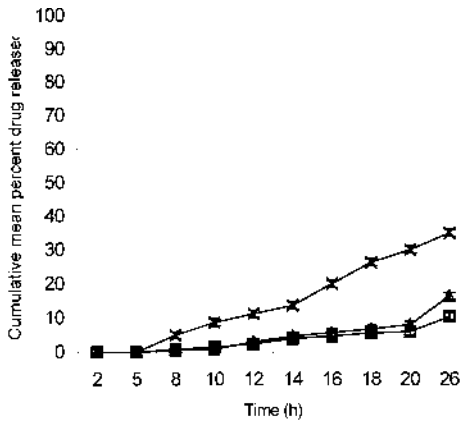

Fig. 1. Cumulative Mean (±S.D.) Percent Drug Released from Mesalazine Tablets (n=3) Compression-Coated Tablet with Different Ratios of Coating Materials Containing 2 : 3, 3 : 2 and 4 : 1 of Locust Bean Gum (LBG) and Chitosan in 0.1 N HCl (2 h), pH 7.4 Buffer (3 h) and pH 6.8 PBS (21 h)

220 mg LBG and 330 mg chitosan: □, 330 mg LBG and 220 mg chitosan: △, 440 mg LBG and 110 mg chitosan: ×.

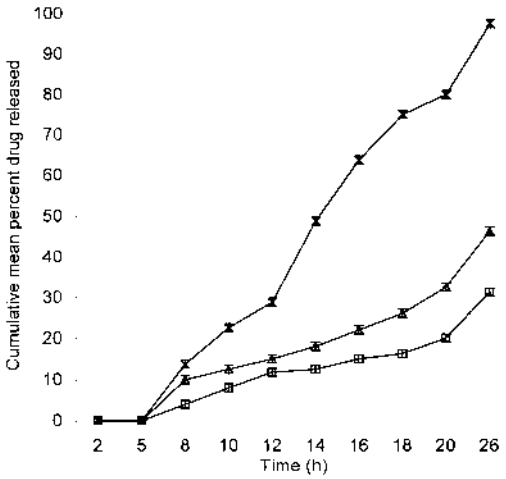

Fig. 2. Cumulative Mean (±S.D.) Percent Drug Released from Mesalazine Tablets (n=3) Compression-Coated with Different Ratios of Coating Materials Containing 2 : 3, 3 : 2 and 4 : 1 of LBG and Chitosan in 0.1 N HCl (2 h), pH 7.4 Buffer (3 h) and pH 6.8 PBS Containing 4% w/v Rat Caecal Contents (21 h)

220 mg LBG and 330 mg chitosan: □, 330 mg LBG and 220 mg chitosan: △, 440 mg LBG and 110 mg chitosan: ×.

Table 2. Pharmacokinetic Parameter of the Mesalazine Compression-Coated Tablet Obtained from the *in Vivo* Studies Carried out Using a 9×3 Complete Cross Over Study in Healthy Human Volunteers

Parameter	F1	F2	F3
C_{max} (μg/ml)	6.78±0.01	13.92±0.49	28.25±0.15
T_{max} (h)	10±0.35	13±0.13	16±0.72
K_{el} (h)	0.07±0.01	0.31±0.01	0.05±0.01
$t_{1/2}$ (h)	10.03±0.5	22.56±0.5	15.03±0.5
$AUC_{0\to t^*}$ (μg·h/ml)	85.24±0.10	196.05±0.11	498.62±0.1
$AUC_{0\to\infty}$ (μg·h/ml)	119.11±0.26	347.22±0.01	1111.42±0.67
$AUMC_{0\to t^*}$ (μg·h/ml)	1776.2±0.26	3098±0.01	8379.46±1.15
$AUMC_{0\to\infty}$ (μg·h/ml)	3316.4±0.07	9955.69±0.03	33841.56±0.74
MRT (h)	27.84±0.06	28.67±0.01	34.95±0.02

Each value represents mean±S.D.

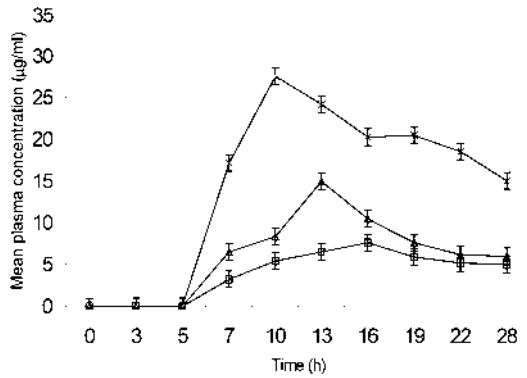

Fig. 3. Mean Plasma (±S.D.) Concentration from Mesalazine Tablets (n=3) Compression-Coated with Different Ratios of Coating Materials 3 : 2, 2 : 3 and 4 : 1 of LBG and Chitosan in Healthy Human Volunteers

220 mg LBG and 330 mg chitosan: □, 330 mg LBG and 220 mg chitosan: △, 440 mg LBG and 110 mg chitosan: ×.

PBS (21 h) are shown in Fig. 1. The results of the drug release studies carried out in the presence of 4% w/v rat caecal contents in pH 6.8 PBS are shown in Fig. 2.

From the above mentioned figures, it was clear that the drug was not released till 5 h, which indicated that the drug was not released in the presence of the 0.1 N HCl and pH 7.4 phosphate buffer.

***In Vivo* Drug Release Studies** The blood samples collected from the healthy male volunteers showed different pharmacokinetic parameters for the different formulations. The parameters are shown in Table 2, and the mean plasma concentration of the different formulation are given in Fig. 3.

The pharmacokinetic parameters and the mean plasma concentration showed that the drug from all the formulation was released only after 5 h and the formulation F3 showed better release.

Discussion

The successful delivery of drugs to the colon requires the protection of drug from being released in stomach and small intestine. In the present investigation, locust bean gum and chitosan at the ratio of 2 : 3, 3 : 2 and 4 : 1 was applied over mesalazine core tablet and drug release studies were carried out under condition mimicking mouth to colon transit.

The *in vitro* release of the drug from tablets coated with coat formulations containing 2 : 3, 3 : 2 and 4 : 1 ratio of locust bean gum and chitosan (F1, F2, F3) was not found till 5 h of testing in simulated gastric fluid and intestinal fluids (Fig. 1). But on exposure to the dissolution fluid, the gum got hydrated and formed a viscous gel layer that slowed down further seeping-in of dissolution fluids towards the core tablet. The hydration of coat seemed not to be affected by the pH of the dissolution medium. Thus, locust bean gum and chitosan in the form of coat was capable of protecting the drug from being released completely in the physiological environment of stomach and small intestine. To assess the integrity of the coats, the drug release were further continued for 21 h by replacing the dissolution medium with pH 6.8 PBS. At the end of the experiment (26 h), the cumulative mean percentage (±S.D.) of drug released from coat formulations F1, F2 and F3 were found to be 10.37±0.60, 16.374±0.22, 35.0±0.28, respectively. This indicated that gum coat would not permit the release of the bulk of the drug until the coat was broken.

The aim of the drug delivery system targeted to the colon is not only to protect the drug from being released in the physiological environment of stomach and intestine, but also to release the drug in the colon after enzymatic degradation of colonic bacteria. Hence, the *in vitro* drug release studies were carried out in pH 6.8 PBS containing 4% PBS of rat caecal contents. At the end of 26 h of testing which included testing in simulated gastric and intestinal fluid, the percent of mesalazine released from the coated tablets with formulation F1 was found to be only 31.25±0.56, and there was no rapid increase in the delivery of drug to the colon. The formulation F2 was found to be 46.25±0.96 and there was a small increase in the release of the drug after 14 h. The formulation F3 showed a rapid increase of the drug form 13 h and the cumulative mean percentage release of the drug at 26 h was 97.5±0.26.

The release rate showed that the coat formulation F3 (4 : 1 ratio of locust bean gum and chitosan) produced better release of mesalazine. About 97.5% of the drug was released in the colon after protecting drug from the stomach and small intestine. It was also evident from the results of drug release in the presence and absence of rat caecal contents that the maximum amount of the drug release occurred by the degradation of the coat material by the enzyme present in the caecal content.

Even though the *in vitro* studies had revealed that the better release was obtained from the coat formulation F3, the *in vivo* studies using human volunteers was ultimate requirement to establish their credibility. The pharmacokinetic parameter and mean plasma concentration (Table 1, Fig. 3) showed that the drug was released only after 5 h indicating that the coat formulations (F1, F2, F3) has a capability of preventing the drug release in the stomach and intestine. It was also indicated that the *AUC* of the formulation F3 was greater (1111.92±0.67 μg·h/ml) when compared to other formulation.

In vitro drug release studies and the *in vivo* studies using the formulation F1, F2 and F3 clearly indicated that the locust bean gum and chitosan as a coat material applied over the core tablet was capable of protecting the drug from being released in the physiological environment of stomach, small intestine and was susceptible to colonic bacterial enzymatic actions with resultant drug release in the colon. Thus, the

study clearly indicated that the locust bean gum and chitosan was a potential colon specific drug delivery carrier in which the 4 : 1 ratio proved itself a good carrier.

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