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In vivo evaluation of time and site of disintegration of polysaccharide tablet prepared for colon-specific drug delivery

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Abstract

Compression coating has been found to be useful for colonic drug delivery. The aim of the present investigation was to evaluate a formulation with a considerably reduced coat weight and gum concentration for colonic drug delivery in vivo using gamma scintigraphy. In vitro studies have found this formulation to be useful for delivery of 5-fluorouracil to the colon. Rapidly disintegrating core tablets containing ^{99m}Tc-DTPA were prepared and compression coating with 150 mg of granules containing a mixture of xanthan (XG), guar gum (GG) and starch. The ratios of the two gums XG:GG in the coat was kept 10:20. In vitro dissolution studies on XG:GG::10:20 tablets containing ^{99m}Tc-DTPA were carried out in simulated upper GIT conditions and also in presence of colonic contents. Cumulative percent release of technetium in the upper GIT conditions and transit time amounted to 4%. The total amount of technetium released in the 24 h of the dissolution study was $53 \pm 3.23\%$. Upon introduction of cecal content into the dissolution medium (4%), the release of technetium from the compression-coated tablet increased to $78.34 \pm 5.34\%$. Gamma scintigraphy studies carried out in six healthy human volunteers showed that the tablet remained intact during its transit through the upper GIT. The anatomical site of disintegration was found to be the ascending colon/hepatic flexure and the disintegration of the tablet started between 4 and 6 h post-dose in all the volunteers with a further spread of tracer into the ascending, transverse, descending and sigmoidal colon.

Keywords: Gamma-scintigraphy; Xanthan gum; Guar gum; Starch; Colon-specific drug delivery

1. Introduction

Colon-specific drug delivery holds promise for direct, more effective delivery of therapeutic agents to the colon for patients being treated for illnesses, such as irritable bowel syndrome (IBS), colonic cancer, ulcerative colitis, Crohn's disease, etc. These delivery systems when taken orally, allow drugs to bypass premature release/absorption in the stomach and the small intestine and release the drug from the delivery system once the delivery system arrives into

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the colon. These delayed release mechanisms are designed to improve the efficacy of the drug by concentrating the drug molecules where they are needed the most, and also minimize the potential side effects and drug instability issues that are frequently associated with premature release of drug in the upper parts of the GIT, namely the stomach and small intestine. Colonic drug delivery may additionally hold potential for oral delivery of relatively fragile peptide drugs, such as insulin, which are delivered by injections (Kinget et al., 1998).

Colon cancer is the second leading cause of cancer deaths. Stage II or III colorectal cancers are treated with surgery alone or with surgery and radiation therapy. Such cases may still experience a cancer recurrence later. Administering chemotherapy after surgery helps reduce the risk of developing a cancer recurrence. Currently, the standard first-line treatment for patients diagnosed with metastatic colorectal cancer is 5-fluorouracil-based chemotherapy (Calabresi and Chabner, 1992). However, this therapy is given parenterally since when given orally, the bioavailability is erratic. Also parenteral administration leads to a number of side effects, which are associated with the distribution of the drug to the non-target sites. Targeted delivery of 5-fluorouracil would not only reduce systemic side effects, but also provide an effective and safe therapy for colon cancer with reduced dose and reduced duration of therapy.

Compression coated systems have been formulated by many research groups. However, a higher coat weight has been used for the purpose (Krishnaiah et al., 1999). In the present study, a compression coated dosage form was designed for colon-specific delivery of 5-fluorouracil using a lower coat weight (Sinha and Kumria, 2003). The compression coat of 150 mg consisted of xanthan gum, guar gum and starch. Xanthan gum (XG) is known to have a greater drug-release retarding property (Talukdar and Kinget, 1995; Sujjaareevath et al., 1998) and synergistically enhanced gel properties in presence of galactomannan gums like guar (Melia, 1991). There are many reports of the use of guar gum for colon targeting. Guar gum (GG) alone has earlier been used in colon-specific drug delivery as matrix-forming material and as a compression coat (with a much higher coat weight) (Wong et al., 1997; Rama Prasad et al., 1998; Krishnaiah et al., 1999). Starch is another polysaccharide, which consists of amylose and amylopectin as constituents. Amylose is known to be degradable in the tracts of colon (Milojevic et al., 1996).

Earlier studies conducted by our research group on the suitability of this compression coat for colon-specific delivery of 5-fluorouracil to the colon showed promising results in vitro in case of XG–GG combination with a XG:GG ratio of 20:10 and 10:20 (Sinha and Kumria, 2003). As xanthan gum is a polysaccharide which is stable to enzymatic hydrolysis, a dosage form for in vivo studies was selected, which contained a minimum percentage of XG, i.e., XG:GG::10:20. Another observation made in the in vitro study was that the compression coat was intact even after 24 h of dissolution and formed a viscous gel around the tablet.

In order to access the suitability of this compression coat to deliver drugs specifically to the colon, a compression-coated system was designed making use of Technetium-DTPA (^{99m}Tc-DTPA) in the core tablets instead of 5-fluorouracil. The release of the tracer from the compression-coated tablets was initially studied under simulated GIT conditions and in presence of colonic contents. Further studies were carried out in healthy human volunteers. The time and site of disintegration of the γ-emitting tablets was traced using a gamma camera.

2. Material and methods

2.1. Materials

Guar gum (M.W. 220,000) was procured from Himedia Laboratories Limited, India. Xanthan gum, USNF and was obtained as gift sample from Dabur Research Foundation, Ghaziabad, India. Cross PVP was obtained as a gift sample from ISP Technologies Inc., USP. ^{99m}Tc-DTPA was obtained by eluting molybdenum obtained from Amersham, London. Maize starch, talc and magnesium stearate used for the preparation of tablets were of pharmacopoeial grade. Scintigraphs were taken periodically using E-cam (Siemens), images stored using an online computer and the digital data analysed later.

2.2. Preparation of granules for compression coating

Granules for the compression coat were prepared consisting of xanthan gum (10%, w/w), guar gum (20%, w/w) and starch (60%, w/w). The ingredients in the quantities mentioned were wet granulated using starch paste (10%). Granules of the above wet mass were prepared by passing through a sieve with a nominal aperture of 1 mm. The granules were dried for 6 h at a temperature of 50 °C. The dried granules were passed through a sieve with a nominal aperture of 1 mm and mixed with talc (1.7%) and magnesium stearate (1.2%). These granules were stored till used.

2.3. Preparation of core tablets for scintigraphic studies

Rapidly disintegrating core tablets containing, ^{99m}Tc-DTPA (1.5 MBq) (absorbed on dried sodium chloride) and a super-disintegrant polyvinyl pyrrolidone (cross-PVP) (2 mg) were prepared. Quantity weighing 52 mg was taken and compressed individually into tablets using 5.1 mm flat plain punches on a single-punch tableting machine (Modern Engg. Works, New Delhi, India) using 4000 kg compression force. Technetium was obtained as a saline solution and a volume containing 1.5 MBq of technetium was evaporated to dryness and mixed with dummy core material and compressed into core tablet. The prepared tablets were compression coated with XG:GG::10:20 combinations.

2.4. Preparation of compression coated tablet

Hundred and fifty milligrams of compression coating material were applied over the core tablets. For compression coating, 43.33% of coat weight was placed in the die cavity followed by carefully centering the core tablet and addition of the remainder of coat weight. The coating material was compressed around the core tablet at an applied force of 5000 kg using 8.2 mm round concave punches using a single-station tableting machine.

2.5. Drug release studies

The ability of the prepared γ-emitting tablets to retard drug release in the physiological environment of the stomach and the small intestine, and release the drug in the colon was assessed by conducting drug release studies in simulated stomach and small intestinal pH, respectively. The changing pH media, Method 1, USP 23, for delayed release tablets was used. Dissolution test was conducted in USP 1 apparatus at 75 rpm and a temperature of 37 ± 0.5 °C. Initial drug release studies were conducted in 750 ml of 0.1N HCl for 2 h. Then, 250 ml of 0.2 M trisodium phosphate was added to the dissolution media and the pH adjusted to 6.8. Samples of the dissolution media were withdrawn after regular intervals of time and γ-emission measured.

2.6. Preparation of rat cecal content medium

Wistar rat weighing 150–200 g and maintained on a normal diet (soaked gram) were used. Forty-five minutes before the commencement of drug release studies, seven rats were killed by spinal traction. The abdomen were opened, the cecae were traced, ligated at both the ends, dissected, and immediately transferred into pH 6.8 buffer previously bubbled with nitrogen. The cecal bags were opened, their contents were individually weighed, pooled, and suspended in the buffer continuously bubbled with nitrogen. These were finally added to the dissolution media to give a final cecal dilution of 4% (w/v). All the above procedures were carried out under nitrogen in order to maintain anaerobic conditions.

2.7. Drug release studies in presence of cecal content

Drug release studies in the cecal content were also carried out using USP dissolution test apparatus as mentioned in the Section 2.5. However, slight modification in the procedure was done. The experiments were carried out in 250 ml beaker immersed in water maintained in the jars of dissolution test apparatus. Initial studies were carried out in 150 ml of 0.1N HCl (pH 1.2) for 2 h. After this, 50 ml of 0.2 M trisodium phosphate was added to the dissolution media and the pH adjusted to 6.8. The study at a pH 6.8 was continued for 3 h after which, cecal content equivalent in cecal content to 8 g were added to 200 ml of buffer (pH 6.8) to give a final cecal dilution of 4%. Dissolution in the cecal content media was carried out till completion of 24 h. The experiments in cecal content media were car-

Table 1
Table showing the site and time of disintegration of the compression coated tablet

Volunteer	Site of initiation of disintegration of the tablet	Post-dose time of initiation of disintegration of the tablet (h)	Post-dose time of complete disintegration of the tablet
1	Hepatic flexure	4.30	12
2	Hepatic flexure/ascending colon	5.00	18
3	Hepatic flexure/ascending colon	5.30	14
4	Hepatic flexure/ascending colon	4.20	13.25
5	Hepatic flexure	6.00	14.5
6	Ascending colon	6.0	12.5

ried out in presence of a continuous supply of nitrogen. At different time intervals, 1 ml sample was withdrawn from the dissolution medium and 1 ml of cecal content (4%), maintained under anaerobic conditions, was replenished into the dissolution media. The sample was placed into the gamma counter and the amount of tracer in the sample was measured. A suitable correction for the decay of tracer was applied.

2.8. Human scintigraphy studies

Six healthy volunteers aged 25–36 years, all non-smokers, were recruited for the study. Volunteers were not taking any medication at the time of the study and all abstained from alcohol for 24 h prior to dosing and throughout the study period. The study was approved by the Institutional Ethics Committee, PGIMER, India. The total effective radiation dose was 1.5 MBq for each subject. Prior to recruitment for the study, the nature of the study was explained to all the volunteers, and each volunteer provided a written consent.

After fasting from midnight, a ^{99m}Tc-DTPA-labeled compression coated tablet was administered to each volunteer with 250 ml water. Anterior and posterior scintigraphic images of 60 s duration were recorded. Images were recorded every 20–30 min using external markers to locate the site of disintegration. During the study, volunteers remained moderately active, and all images were acquired with volunteers lying down between the anterior and posterior cameras. The images were recorded using a Bartec computer system, and were stored via an online computer system for subsequent analysis.

On the day of the study, all volunteers consumed a standard diet. Subjects had previously been instructed to fast overnight (from midnight) on the evening prior to dosing. The breakfast was given 2 h post-dose and consisted four slices of bread, 50 g jam, 50 g cheese and a cup of tea/coffee. This was followed by a standard lunch 5 h post-dose. This consisted of a medium-sized dosa, curry, sauce and tea/coffee. Post-lunch, the volunteers were allowed fluids ad libitum. Tea was given 7 h post-dose. A standard dinner was given 10 h post-dose, which consisted of vegetable (one bowl), pulses (one bowl), curd (one bowl) and chapatis (four medium sized).

The data were analysed to provide information regarding the time and site of disintegration of the prepared compression coated tablet. The site and time of initial disintegration of the compression-coated tablet was identified and is listed in Table 1. Complete disintegration was defined as the time of complete dispersion of the core contents.

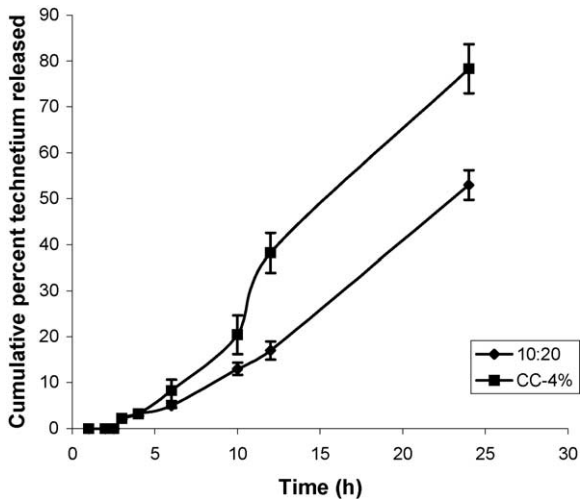

Fig. 1. In vitro technetium release studies from compression coated tablets XG:GG::10:20 with 150 mg coat weight in dissolution medium in absence and in presence of cecal content (CC 4%) ($n = 3$).

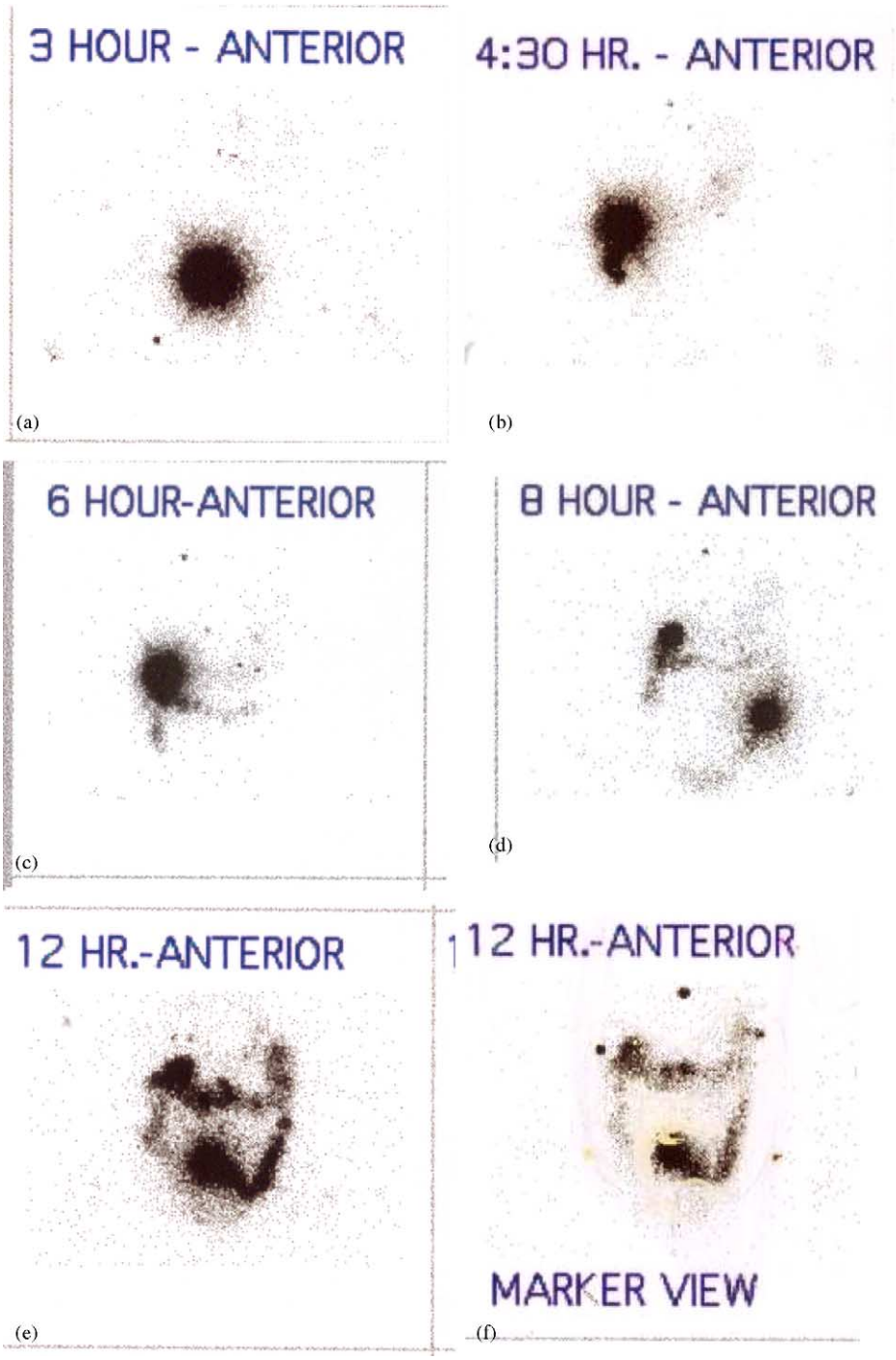

Fig. 2. Gamma scintigraphy showing the tracer at (a) 3 h showing the intact tracer-containing core somewhere in the small intestine, (b) 4 h showing the initiation of disintegration of the tracer-containing core from the hepatic flexure with the spread in ascending colon/transverse colon, (c) 6 h showing the disintegration of the tracer-containing core from the hepatic flexure with the spread in ascending colon/transverse colon, (d) 8 h showing the disintegrated tablet core with two distinct portions, one each in the hepatic flexure and the descending colon, (e) 12 h scintigraph with the spread of the tracer all along the ascending, transverse, descending and sigmoidal colon, and (f) 12 h scintigraph with the site of disintegration, i.e., the colon distinguished with the help of external markers (volunteer 1).

3. Results and discussion

3.1. In vitro release studies of technetium from the compression coated XG:GG::10:20 tablets in the presence and absence of cecal content medium

In vitro release studies of the tablets containing technetium in the core tablets showed that the release of technetium from the core tablets takes place at a highly retarded rate. Cumulative percent release of technetium during the initial 5 h (2 h at a pH 1.2 followed by 3 h at a pH 6.8) of the study amounted to 4%. The total amount of technetium released in the 24 h of the dissolution study was $53 \pm 3.23\%$. Upon introduction of cecal content into the dissolution medium, the release of technetium from the compression coated tablet increased. The total amount of technetium released in the 24 h of the dissolution study in presence of cecal content was found to be $78.34 \pm 5.34\%$, thus, showing the potential of the compression coat of XG:GG::10:20 (150 mg) to undergo degradation in the presence of colonic contents, and thus their ability to release the drug-containing core into the tracts of the colon (Fig. 1).

3.2. In vivo performance of the tablet

The recorded time for onset of disintegration and the time for complete tablet disintegration were taken as the mid-time between the times recorded for the two images about the transition. The data showing the time and site of disintegration of the tablet has been shown in Table 1. In all the volunteers, tablet integrity was maintained whilst the preparation resided within the stomach and the small intestine indicating that the coat consisting of xanthan gum, guar gum and starch is capable of protecting the core from being released. In all the volunteers, the initial signs of disintegration were observed while the tablet had arrived into the colon. The time of disintegration of the tablet varied from 4.2 to 6.0 h. The anatomical location of disintegration was found to vary between the ascending colon to the hepatic flexure. The site of disintegration was evaluated making use of external markers on the abdomen of the volunteers. On entering the colon, the ^{99m}Tc-DTPA core tablets compression coated with XG:GG::10:20 coat began to release the tracer in all the six volunteers and resulted in the distribution of the released tracer throughout the colon (Fig. 2a, b, c, d and e).

The biodegradation of guar gum in the form of matrix tablets and in the form of compression coat has been established (Krishnaiah et al., 1998, 1999). Though there is no direct evidence for the degradation of guar gum and starch containing compression coat by the enzymes of colonic bacteria, the absence of release of the tracer in the stomach and the small intestine, and the release of the tracer soon after entering the colon indicated degradation of the hydrated gum containing coat by the colonic bacteria, thereby exposing the soluble coat containing a super disintegrant. Earlier studies in simulated gastric, intestinal and colonic fluids have indicated that the coat consisting of xanthan gum, guar gum and starch would allow the release of the drug from the core at a highly retarded rate, unless the coat is degraded by the colonic bacteria (Sinha and Kumria, 2003). Presence of xanthan gum in presence of guar gum would allow formation of a thicker viscous gel layer around the surface of the tablet (as compared to while using guar gum alone) on being exposed to the fluids of the GIT. This viscous layer retards seeping of the fluids into the core.

Similar studies making use of a compression coat of guar gum alone as a compression coat have been carried out earlier (Krishnaiah et al., 1999). But the presence of xanthan gum in the coat will help form more thicker gels (at a lower gum content) around the core which, in turn, will reduce the gum content of the coating material (making processing easier). Thicker gels will reduce the diffusion of drug from the core to negligible levels. Additionally, xanthan gum being a higher swelling gum will lead to higher swelling of the coat, thereby giving greater surface area for the action of hydrolytic enzymes in the colon. Presence of a mixture of two degradable polysaccharides, namely guar and starch in the compression coat would increase the susceptibility of the coat to a wider range of bacterial population.

4. Conclusion

Gamma scintigraphy studies conducted on six healthy volunteers validate the design concept behind the release mechanism using the compression coat of 150 mg containing xanthan gum and guar gum in the

concentration of 10:20 with starch constituting the main filler in the compression coat. The scintigraphs confirm the design concept, in that the tablet does not disintegrate, and hence release doesn't start till the tablet reaches the colon. Within the colon, the tablet-containing core gets disintegrated and the spread of the tracer from the tablet is observed. In all the volunteers, the initiation of disintegration of the tablet could be observed after a time lag of 4–6 h. The anatomical site of disintegration of the tablet was ascending colon/hepatic flexure.

The human studies provide in vivo 'proof of concept' for colonic delivery strategy while underlining the use of scintigraphy in product development for colon targeting.

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