The Use of Scintigraphy to Provide "Proof of Concept" for Novel Polysaccharide Preparations Designed for Colonic Drug Delivery

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Purpose. The aim of the present study was to provide "proof of concept" data in man for novel polysaccharide preparations designed for colonic drug delivery using gamma scintigraphy.

Methods. Two placebo calcium pectinate matrix tablet formulations were studied: one contained calcium pectinate and pectin (CaP/P) and was designed to rapidly disintegrate in the ascending colon, the other contained calcium pectinate and guar gum (CaP/GG) and was designed to disintegrate more slowly, releasing its contents throughout the ascending and transverse colon. Both formulations were enteric coated in order to protect them from the stomach. Ten healthy volunteers received either a CaP/P or CaP/GG tablet, in a randomised cross-over study. Transit and disintegration of the radiolabelled formulations was followed by gamma scintigraphy. Rat studies were conducted in order to verify that the expected colonic degradation of the polysaccharide formulations was as a consequence of bacterial enzyme attack.

Results. The *in vivo* clinical study confirmed the results obtained in the rat and bench *in vitro* fermentation models; complete tablet disintegration for Formulation CaP/GG appeared to be slower than that of Formulation CaP/P and the time and the location of complete tablet disintegration was more reproducible with Formulation CaP/P compared to Formulation CaP/GG.

Conclusions. These results provide "proof of concept" data for the use of calcium pectinate preparations for drug delivery to the colon and highlight the value of scintigraphy in focusing the development strategy for colonic targeting preparations.

KEY WORDS: calcium pectinate; colon targeting; gamma scintigraphy; "proof of concept."

INTRODUCTION

Colonic drug delivery is intended for the local treatment of ulcerative colitis, irritable bowel syndrome and can potentially be used for colon cancer or the systemic administration of drugs that are adversely affected by the upper gastrointestinal tract (1). The advantages of local treatment in the colon are: reduced incidence of systemic side effects, lower doses of drug can be utilized, and maintenance of the drug in its intact form as close as possible to the target site. The colon has also been mentioned as an ideal site for protein and peptide absorption (2). Acidic and enzymatic degradation are major obstacles in the oral administration of peptide drugs (3), but by targeting delivery to the colon it is anticipated that proteolysis can be minimized (4).

There has been considerable research into the design of colonic delivery systems and targeting has been achieved in several ways: by coating drugs with pH sensitive polymers (5), by coating drugs with bacterial degradable polymers (6), using prodrugs (7) and delivering drugs through bacterial degradable matrices (8–10).

The microbiological approach to colon targeting relies on the ability of the colonic microflora to degrade various types of substrate that escape small bowel digestion (1). Pectins are polysaccharide components of plant primary cell walls and consist of linear polymers of D-galacturonic acid residues with varying degrees of methyl ester substituents. Pectins are broken down by various microbial sources including human colonic bacteria (11) and may therefore be utilized as colonic delivery systems if their water solubility is reduced. This can be accomplished by crosslinking with calcium to produce an insoluble pectinate gel (9). It has been previously shown that calcium pectinate (CaP) tablets, containing indomethacin as a drug marker, degraded and released their drug load to a greater extent in the presence of pectinolytic enzymes or rat cecal contents than in phosphate buffer (9).

Two calcium pectinate (CaP) matrix formulations have been developed as potential colon targeting systems. One formulation contains calcium pectinate and pectin (CaP/P) and is designed to rapidly disintegrate in the ascending colon. The second formulation contains calcium pectinate and guar gum (CaP/GG) and is designed to disintegrate more slowly that (CaP/P), releasing its contents throughout the ascending and transverse colon. Both formulations are enteric coated so that the tablet matrices are protected from the harsh environment of the stomach.

In the early stages of development of novel colonic delivery systems, considerable time can be lost in establishing the likely potential of any given research strategy because of a lack of totally predictive *in vitro* or animal models. Pilot pharmacokinetic studies in small groups of healthy subjects provide unsuitable end points for the assessment of colonic targeting preparations (12). However, scintigraphic evaluation provides detailed information on the *in vivo* performance of novel oral formulations and can be used to focus the product development process (13). Therefore the objective of this scintigraphic study was to provide "proof of concept" data for novel polysaccharide preparations designed for colonic drug delivery.

MATERIALS AND METHODS

CaP Tablets

Two types of CaP tablets were manufactured. The first contained pectin (formulation CaP/P) and the second contained guar gum (GG, formulation CaP/GG) as binding agents, both selected because of their reported degradation by human colon bacteroides (14). Prior to tableting each formulation was mixed with the isotopically enriched stable isotope samarium oxide (^{152}Sm) (2mg/tablet). Both formulations were enteric coated with Eudragit L, which was applied by spray coating the aqueous

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dispersion of Eudragit L in a pan coater. The integrity of the enteric coating was demonstrated *in vitro* prior to the scintigraphic study, by a dissolution study in simulated gastric buffer.

In Vivo *Degradation Analysis in the Rat*

Pre-weighed tablets were tested for biodegradation in the cecum of Sabra rats using a previously described method (15). The tablets were mounted in gauze bags that were implanted, each into the cecum of an anaesthetised rat (a single dosage form/rat). The rats were allowed to recover and kept on a normal diet for 2 days, after which they were sacrificed and the bags opened. In the bags where tablet residues were found, residues were collected and dried until no further weight loss. Sabra rats which were treated with an antibiotic cocktail (300 ml of intra-cecal administration of ampicillin 250 mg/ml, chloramphenicol 0.5 mg/ml and cefazolin 250 mg/ml) were used as controls. In the "antibiotics" study, tablets of the same size were weighed, implanted and the residues dried and weighed after 2 days. The antibiotic cocktail was also added to the drink water of the control rats for the 2 days of the study.

Scintigraphic Evaluation of Formulations CaP/P and CaP/GG in Man

Tablets Radiolabelling

Both tablet formulations (9 mm diameter) were radiolabelled by neutron activation. This technique involves the incorporation of a small amount (2 mg) of the isotopically enriched stable isotope samarium oxide (^{152}Sm) into the formulation prior to manufacture. The stable marker is then converted to the radioactive isotope (^{153}Sm) by exposing the dosage form to neutron bombardment. The tablets in this study were irradiated for six minutes in a neutron flux of 10^{12}n cm^{-2}s^{-1}, 48 hours prior to dosing. The *in vitro* tablet formulations had sodium salicylate incorporated into them as a drug marker. Dissolution studies using simulated intestinal buffer were carried out both before and after the irradiation of the sodium salicylate formulations and showed that there was no difference between the drug dissolution profiles. Therefore indicating that the neutron activation process had no significant effect on the dosage forms. The tablets were assayed for radioactive content on the morning of each study day and, at the time of administration each tablet contained approximately 1MBq of ^{153}Sm.

Subjects

This was an single blind randomised cross-over study carried out in ten healthy volunteers (7 male, 3 female) aged between 21 and 52 years. Each volunteer was examined by a physician before the study and was judged to be in good health on the basis of medical history, physical examination and routine laboratory data. The clinical protocol was approved by an independent Ethics Committee and approval for administration of radiolabelled preparations was obtained from the Department of Health, London. Each subject provided written informed consent to participate in the study.

Study Protocol

The volunteers arrived fasted (from midnight) at the study site. Anterior anatomical markers containing 0.1 MBq^{99m}Tc were taped to the skin over the right lobe of the liver, in the same transverse plane as the distal end of the oesophagus, of each subject. One radiolabelled CaP/P or CaP/GG tablet was administered to each of the volunteers at approximately 8:00 am with 200 ml of water.

Anterior scintigraphic images, each of 50 seconds duration were taken using a gamma camera (General Electric Maxicamera) with a 40 cm field of view and fitted with a low energy parallel hole collimator. Images were recorded at approximately 10 minute intervals up to eight hours post-dose and thereafter at 15 minute intervals up to 12 hours post-dose. A return visit to the clinical unit was made 24 hours post-dose to allow the acquisition of a final image. The volunteers remained moderately active during the study period and all images were acquired with the subjects standing in front of the gamma camera. The images were recorded using a Bartec computer system and were stored on optical disk for subsequent analysis. A standard light lunch and dinner were provided at 4 hours and 9 hours post-dose, respectively. Volunteers drank 200 ml of water 2 hours post-dose and then drinks were allowed *ad libitum* after lunch.

Data Analysis

The data were analysed to provide information on gastric emptying time, small intestinal transit time, colon arrival time and the time and position of both initial and complete tablet disintegration. The gastrointestinal transit of the tablet formulations was calculated using a previously validated procedure (16). Initial tablet disintegration was defined as the time taken to detect signs of release of radioactive marker from the tablet in consecutive images whilst complete disintegration was defined as the time at which all the radiolabel had dispersed within the gastrointestinal tract and no signs of a distinct 'core' remained.

RESULTS

Degradation Studies

The degradation of the CaP tablets as measured after implantation in the rat cecum is shown in Figure 1. Two days

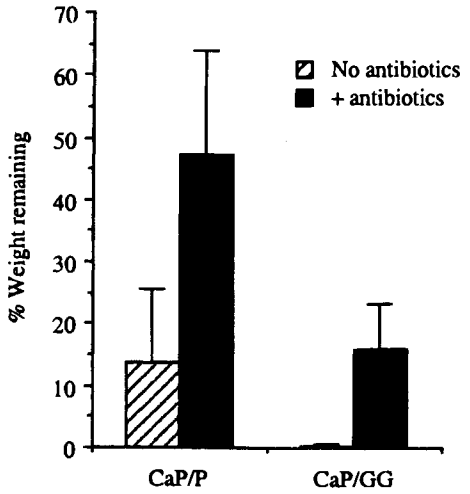

Fig. 1. The degradation of two CaP tablet formulations (CaP/P and CaP/GG) in the rat cecum with and without antibiotic treatment. Shown are the average of 4 measurements ± S.D.

Table I. Transit Profile of the Placebo Calcium Pectinate Colonic Delivery System [Formulation CaP/P] (time in minutes)

Subject number	Gastric emptying (time post-dose)	Colon arrival (time post-dose)	Small intestinal transit
1	76	254	178
2	17	254	237
3	16	313	297
4	80	246	166
5	110	261	151
6	22	241	219
7	26	259	233
8	11	243	232
9	58	250	192
10	192	395	203
Mean	61	272	211
SD	57	48	42
Median	42	254	211
n=	10	10	10

in the rat cecum caused 86.2% degradation of formulation CaP/P (52.7% degradation after antibiotic treatment) and almost 100% of formulation CaP/GG (83.9% degradation after antibiotic treatment). Figure 1 shows that although CaP/GG tablets were less water soluble, they degraded almost completely after two days in the cecum of the rat with marked differences between the antibiotic and non-antibiotic groups. The enhanced degradation of this formulation can be attributed to the better swelling properties of GG as compared to pectin (19).

Scintigraphic Studies

Transit data for CaP/P and CaP/GG tablets are provided in Tables I and II, respectively. Tablet disintegration data for CaP/P and CaP/GG tablets are provided in Tables III and IV,respectively.

Table II. Transit of the Placebo Calcium Pectinate Colonic Delivery System [Formulation CaP/GG] (time in minutes)

Subject number	Gastric emptying (time post-dose)	Colon arrival (time post-dose)	Small intestinal transit
1	11	242	231
2	6	250	244
3	41	253	212
4	15	117	102
5	5	250	245
6	17	262	245
7	11	177	166
8	4	189	185
9	4	240	236
10	51	247	196
Mean	17	223	206
SD	16	47	46
Median	11	245	222
n=	10	10	10

DISCUSSION

Degradation Studies

The CaP used in this study was previously shown to degrade enzymatically to produce short chain fatty acids in bench chemostat studies employing human fecal matter. The first stage of the study included implantation of CaP tablets which had been formulated with pectin or guar gum and coated with a thin enteric coating, in the cecum of the rat and *in vivo* analysis of their degradation over 48 hours. The rationale for the use of this kind of model was the wish to mimic, as close as possible, closed colon conditions, since (a) bench chemostat employs fecal matter, which does not necessarily fully represents the real colonic environment loaded with bacterial enzymes, and (b) the rat cecum has been previously reported to possess similar enzymatic activity to the human colon (17).

The significant difference observed between the degradation in antibiotic treated and non-treated rats of the two formulations (CaP/P and CaP/GG), can be explained by the swelling properties of the two tablet formulations. Both formulations contain CaP which swells and therefore is able to absorb enzyme-rich fluids in the colon. The CaP/GG formulation contains guar gum which swells slowly but to a larger extent. As will be seen below, over a period of time of the scintigraphy study (24 hours) the swelling rate of the GG was not sufficient to enhance its extent of degradation. On the contrary, it was believed to create a gel layer which caused it to be the "slow" formulation. However, after 48 hours in the rat cecum the GG swells sufficiently to cause an enhanced degradation of the tablet formulation (Figure 1). Swelling is a major requirement for enzymatic degradation in the colon (15,19). It can be speculated that by balancing the amount of GG in the formulation a degree of control over the degradation kinetics of the tablets can be attained.

Scintigraphic Studies

Gastrointestinal Transit

Two main parameters influence the gastric emptying of pharmaceutical preparations: the physical size of the formulation and whether it is administered in the fed or fasted state. When the stomach is empty of food, i.e., in the fasted or interdigestive state, indigestible solids such as single unit dosage forms will be emptied by the action of the migrating myoelectric complex (MMC). The MMC is usually divided into four phases of activity, which recur approximately every 2 hours. Phase III of the MMC is known as the 'housekeeper wave', and acts to empty the stomach of swallowed saliva, cellular debris and indigestible solids.

The small intestinal transit times for the two formulations are in good agreement with those reported previously for solutions, pellets and tablets of 180 (±60) minutes (18). Tablet stasis occurred at the ileocaecal junction (ICJ) for both formulations in all the volunteers, a phenomenon previously reported in the literature (16).

Tablet Disintegration

Although both formulations showed subtle signs of initial tablet disintegration in the small intestine in all subjects, all

Table III. Disintegration Profile of the Placebo Calcium Pectinate Colonic Delivery System [Formulation CaP/P] (time in minutes)

	Initial Tablet Disintegration			Complete Tablet Disintegration	
number	Time post-dose	Time post-GE	Anatomical position	Time post-dose	Anatomical position
1	176	100	SI	266	AC
2	95	78	SI	$720 < t < 1417$	—
3	163	147	SI	520	AC
4	123	43	SI	707	AC
5	164	54	SI	575	DC
6	51	29	SI	490	AC
7	148	122	SI	714	HF
8	43	32	SI	547	AC
9	96	38	SI	681	AC
10	249	57	SI	547	AC
Mean	131	70		561	
SD	61	41		139	
Median	136	56		547	
n=	10	10		9	

Note: SI = small intestine; AC = ascending colon; HF = hepatic flexure; DC = descending colon; GE = gastric emptying; ITD = initial tablet disintegration.

tablets remained essentially intact during their transit through the small bowel with only a very small amount of disintegration occurring before either of the formulations reached the colon. There appeared to be no difference in initial tablet disintegration time for the two formulations.

Incorporating calcium into a pectin matrix decreases the solubility of the pectin and makes it more susceptible to enzyme attack, however it has been shown that the amount of calcium in the formulation needs to be carefully controlled in order to ensure an optimum delivery system (10). The calcium forms cross links between two pectin molecules in a section on the chain where there are no methoxy groups. As the concentration of the calcium incorporated in a formulation increases, the number of cross links also increases until an insoluble gel is formed. However, if the calcium content is increased above a critical concentration, the polysaccharide gel strength weakens and the formulation disintegrates. Bacterial degradation of a nonstarch polysaccharide like pectin is highly dependent upon its water solubility and therefore any reduction in water solubility greatly affects the degradation rate (19). Also, for microbial attack to occur, the bacterial population has to be able to reach the target site. As was discussed above, the guar gum that is present in the formulation designed for more distal colon delivery (CaP/GG) is thought to be responsible for the slow breakdown of CaP matrix over 24 hours. The rationale behind the formulations was confirmed *in vivo* since complete tablet disintegration for Formulation CaP/GG appeared to be slower than that of Formulation CaP/P; only six of the volunteers showed complete tablet disintegration with Formulation CaP/GG within the 12-hour study period compared to nine subjects with Formu-

Table IV. Disintegration Profile of the Placebo Calcium Pectinate Colonic Delivery System [Formulation Cap/GG] (time in minutes)

	Initial Tablet Disintegration			Complete Tablet Disintegration	
Number	Time post-dose	Time post-GE	Anatomical position	Time post-dose	Anatomical position
1	124	113	SI	547	AC
2	127	121	SI	519	HF
3	116	75	SI	$719 < t < 1406$	—
4	92	77	SI	622	DC
5	51	46	SI	447	DC
6	123	106	SI	$718 < t < 1474$	—
7	68	57	SI	561	AC
8	145	141	SI	$720 < t < 1427$	—
9	57	53	SI	$720 < t < 1434$	—
10	135	84	SI	621	AC
Mean	104	87		553	
SD	34	32		66	
Median	120	81		554	
n=	10	10		6	

Note: SI = small intestine; AC = ascending colon; HF = hepatic flexure; DC = descending colon; GE = gastric emptying; ITD = initial tablet disintegration.

lation CaP/P. The time and the location of complete tablet disintegration was more reproducible with Formulation CaP/P compared to Formulation CaP/GG.

Although complete tablet disintegration of both formulations occurred in the colon, gamma scintigraphy is unable to establish the exact disintegration mechanism. Therefore, to exclude timed release mechanism, supportive evidence is still required. Implantation of the dosage forms in a cecum of the rat may be a reasonable solution. Assessing the *in vivo* performance of the technology following either fed administration or, alternatively, administering the tablets to subjects with altered gut flora following pre-treatment with antibiotics would allow more realistic assessment of whether or not microbial degradation was responsible for targeting specificity.

In conclusion, the scintigraphic evaluation of the gastrointestinal transit and disintegration of the placebo calcium pectinate formulations highlighted important features about this drug delivery concept. The tablets arrived in the colon essentially intact and complete tablet disintegration occurred in the colon for both formulations in all subjects. Complete tablet disintegration for Formulation CaP/GG appeared to be slower than that of Formulation CaP/P and the time and the location of complete tablet disintegration was more reproducible with Formulation CaP/P compared to Formulation CaP/GG. These results provide "proof of concept" data for the use of calcium pectinate preparations for drug delivery to the colon.

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