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An in vivo investigation into the suitability of pH dependent polymers for colonic targeting

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Summary

The behaviour of rapidly disintegrating tablets coated with a model pH dependent polymer, Eudragit S®, have been investigated in vivo, using gamma scintigraphy, to assess its suitability for use in colonic drug delivery. Studies on human volunteers demonstrated that the polymer is capable of protecting a core tablet through the stomach and upper small intestine. In vivo disintegration was extremely variable in both time (5.0 → 15 h) and position. The disintegration sites varied from the ileum to the splenic flexure, showing a lack of site specificity. Spreading of disintegrated material occurred aborally with no back mixing of colonic contents being observed. This study gives important information as to the design of an optimum colonic drug delivery system.

Introduction

There is increasing interest in the targeting of drugs to the colon using the oral route. One approach to targeting has been to exploit pH differences between the colon and the remainder of the gastro-intestinal (GI) tract. Recent in vivo studies using pH telemetry capsules (Evans et al., 1988) have provided evidence of variability in pH in specific regions of the GI tract and a fall in pH from the ileum to the colon. Using these measurements as a basis, Ashford et al (1992) conducted in vitro dissolution and disintegration studies on tablets coated with a pH dependent polymer, Eudragit S®, as a model. The results demonstrated that release of drug from the tablets was variable. Theoretically in extreme cases, they indicated that disintegration could possibly occur as high as the duodenum or not at all. This paper reports the results of a gamma scintigraphic investigation into the disintegration of tablets coated with a pH dependent polymer and provides an insight into the behaviour of such dosage forms in the colon.

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Materials and Methods

Preparation of radiolabelled coated tablets

The radiolabelled tablets were designed to be as similar as possible to the salicylic acid core tablets used in the previous study (Ashford et al., 1992). Diethylenediaminepentaacetic acid (DTPA) was labelled with ^{99m}Tc using a standard test kit. This radiolabelled solution was mixed with sodium chloride solution (80 mg sodium chloride in 2 ml saline) on a vortex mixer and then evaporated by blowing air over the solution in a boiling water bath to leave a solid containing ^{99m}Tc-DTPA. The sodium chloride was added to 'bulk out' the radiolabelled compound. A small quantity of this material was mixed, by hand, for 5 min with 340 mg of excipients comprising 20% w/w Avicel, 1% w/w magnesium stearate and 79% w/w Fastflo lactose. The resulting mixture was compressed using a hand hydraulic press (Beckman, Model P16) and 10 mm bi-convex punches under a compression force of 4000 kg. Three radiolabelled tablets were prepared for each volunteer. The dimensions and weights of the tablets were recorded. These tablets together with 97 core salicylic acid tablets, were coated with Eudragit S (Rohm Pharma, GmbH) as described previously (Ashford et al., 1992).

A coat giving a 5% weight increase on coating was applied. The radiolabelled coated tablets were detected using a Geiger counter and separated from the rest of the batch. The coats were left to cure for at least 16 h under ambient conditions. The process was repeated for each volunteer.

In vitro testing of radiolabelled coated tablets

Due to the nature of the in vivo studies, each radiolabelled tablet had to be prepared separately. Therefore considerable in vitro testing was carried out to check the integrity of the coating process and inter- and intra-batch variability. This testing was performed on the salicylic acid tablets used to bulk out the radiolabelled tablets during the coating process.

The weights and thicknesses of the tablets ($n = 20$) were recorded so that the amount of coat applied could be estimated. Six tablets from each batch were subjected to 6 h of disintegration testing in 0.1 M HCl using the B.P. 1988 apparatus without discs. If the tablets did not remain intact the batch was discarded.

Dissolution studies using a flow through dissolution apparatus (Dissotest, Sotax AG, Switzerland) were carried out on the salicylic acid tablets coated together with each batch of radiolabelled tablets. The dissolution medium was 0.2 M mixed sodium phosphate buffer at pH 7.5. Because the dissolution apparatus had six cells six out of the seven batches were compared in a blocked design dissolution experiment based on a latin square. Six tablets from each batch were distributed in a regular manner, i.e., blocked between the six dissolution cells in six experimental runs. A three-way analysis of variance was performed on selected parameters (lag time before disintegration, taken as the intercept of the straight line portion of the release curve with the time axis and time for 50% dissolution; Ashford et al., 1992). Four variances were estimated, representing the differences between batches, between cells, between runs and the residual variance which represents the random variation between tablets in the same batch. The seventh batch was tested independently.

In vivo studies

Images of the radiolabelled tablets in vivo were obtained using a Scinticamera NE 8960 (Nuclear Enterprises, U.K.) with a 40 cm field of view and fitted with a low energy (140 keV) parallel hole collimator. Seven healthy volunteers of mean age 27 years (range 20–48 years) participated in the study. The purpose of the study was fully explained and each had given their informed consent. The study was approved by the University of Manchester Ethics Committee.

Each volunteer fasted for at least 10 h prior to the study. This requirement was firstly to attempt to standardise the conditions of gastric motility and secondly to increase transit to the lower gastro-intestinal tract; the fasted stomach is likely to empty a large single unit faster than the fed stomach. Two external markers containing a point source of ^{99m}Tc encased in lead and insulating tape (to protect the body) were attached on ei-

ther side of the body in a position approximately corresponding to the left lobe of the liver. These markers allowed correct alignment of the volunteer during successive images.

On the morning of the study, the activity of the tablet was checked to be approx. 3 MBq using an isotope assay calibrator (Type 238, D.A. Pitman Ltd, U.K.). Each volunteer stood in front of the camera and swallowed the tablet with 50 ml of orange juice, a volume that was considered sufficient to ensure that the tablet passed directly into the stomach. No food or drink was permitted until the tablet had emptied from the stomach when a breakfast of toast, butter and marmalade and either tea or coffee was given. All the volunteers ate a similar sandwich lunch and drank orange juice, tea or coffee as requested during the study.

Anterior images were taken almost continuously (10×2 min frames, 2 min rest) until the tablet was observed to arrive in the lower small intestine. On arrival in this region images were taken every 10 min (2 min anterior image, 2 min posterior image, 6 min rest). Follow-up images, each of 5 min duration, were taken the following morning.

By almost continuous imaging, the transit of the intact tablet could be accurately followed. The time taken for gastric emptying, small intestinal transit, residence in the lower small intestine, passage through the ileo-caecal junction and ascending colonic transit of the intact tablet were recorded where applicable. The point and time of tablet disintegration was recorded. Where possible, two regions of interest were drawn, one around the tablet and the other around its released activity. Geometric mean counts were calculated and in vivo release curves constructed.

Results and Discussion

Considerable effort was made to ensure the similarity of properties of each of the administered tablets, particularly as regards the Eudragit coat. The characteristics of the seven batches used in the gamma scintigraphy studies are presented in Table 1. Only small differences in the % weight increase on coating, estimated mean tablet coat weights (calculated from the difference between the mean coated and core tablet weights) and coating thicknesses (calculated from the difference between the face and edge coated and core thicknesses) of the batches were observed.

TABLE 1

Properties of tablets coated together with radiolabelled tablets

Coated batch no.	% weight increase on coating	Mean coat weight (mg)	Thickness (μm)		
			Face	Edge	Average
G1	5.2	19	95	95	95
G2	5.8	21	80	105	93
G3	5.4	21	80	100	90
G4	5.4	20	90	90	90
G5	4.7	20	65	80	76
G6	4.9	19	65	90	78
G7	5.0	20	65	80	73
Mean $\pm$ SD	5.2 ± 0.4	20 ± 0.8	77 ± 12	91 ± 9	85 ± 9

All tablets ($n = 6$) remained intact after 6 h of disintegration testing in acid, indicating that each tablet was adequately coated. To gain an indication of the reproducibility of the batches, six tablets from six batches were tested in a blocked design dissolution experiment. The latin square, the release results and the analysis of variance tables for the lag times and $T_{50\%}$ values are presented in Tables 2–5, respectively. The variances between cells and runs were small and the F statistics (2.055, 2.286, 1.588 and 1.170) were

TABLE 2

The blocked dissolution experiment to assess inter and intra batch to batch variability

Run	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
1	G1	G2	G3	G4	G5	G6
2	G6	G1	G2	G3	G4	G5
3	G5	G6	G1	G2	G3	G4
4	G4	G5	G6	G1	G2	G3
5	G3	G4	G5	G6	G1	G2
6	G2	G3	G4	G5	G6	G1

G1–G6 refer to tablet batches and correspond to gamma scintigraphy studies 1–6.

TABLE 3

Release characteristics from the blocked dissolution experiment (lag time / $T_{50\%}$ values (in min) – tablets in same positions as in Table 2)

Run	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
1	9.1/10.8	10.5/12.2	8.6/10.8	9.4/10.8	8.6/10.1	9.5/11.1
2	10.5/12.8	9.4/11.2	11.5/13.4	8.5/10.8	11.7/13.7	10.1/11.5
3	8.6/10.3	10.0/17.5	10.4/11.8	11.1/11.3	10.4/11.3	11.1/11.3
4	10.1/11.2	8.2/10.0	11.1/15.7	10.0/11.2	9.3/11.1	10.0/11.1
5	8.2/11.1	9.3/10.4	9.6/11.3	10.0/11.4	10.2/11.4	12.5/14.3
6	11.1/12.8	10.0/11.4	10.1/11.4	8.2/11.4	12.1/13.9	9.6/11.1

less than the critical value at the 1% level (4.100). Therefore, the variations between cells and runs were not statistically significant and were assumed to be negligible. The residual variances which represented the random variation between tablets in the same batch, as well as any imprecision in the analytical method or data processing, were also small which indicated good intra-batch uniformity of the tablet coats as well as adequate data processing. The highest variance for both the lag times and the $T_{50\%}$ values was seen between batches and these were significant at the 1% level when compared to the small residual variance. The mean lag time ($n = 36$) was 9.96 ± 0.8 min (range 8.2–12.5 min) and the mean $T_{50\%}$ ($n = 36$) was 11.80 ± 1.6 min (range 10.1–17.5 min). Therefore, the small differences between the maximum and minimum lag times of 4.3 min and the maximum and minimum $T_{50\%}$ values of 6.4 min are unlikely to affect the in vivo results.

Only six of the seven batches were used in the blocked dissolution design. The mean lag times and $T_{50\%}$ values of the seventh batch (G7) were 10.6 ± 1.0 and 13.3 ± 2.2 min, respectively. Using the Student's t-test to compare the mean and standard deviation of the lag times and $T_{50\%}$ values from batch G7 and the total mean and standard deviation of these parameters from batches G1–G6, it was concluded that batch G7 was not significantly different from batches G1–G6. The t-statistics were 1.473 (lag times) and 2.215 ($T_{50\%}$ values) which were less than the critical t values at the 1% level of 3.707 (degrees of freedom = 6). It was concluded that differences between the in vitro characteristics of the administered tablets were minimal as compared to the intra- and inter-subject variation likely to be found in vivo.

The regularity of imaging allowed the transit of the dosage form to be followed in detail and prevented any information being missed when the tablet was travelling at its fastest. A precise point of disintegration could also be adequately determined. Errors in obtaining quantitative data arise from both attenuation (particularly with low energy emitters such as ^{99m}Tc) and movement of the

TABLE 4

Analysis of variance table for lag times of salicylic acid tablets coated together with radiolabelled tablets

Source of variation	Sum of squares	Degrees of freedom	Mean square	F statistic
Between batches	18.823	5	3.765	6.658
Between cells	5.809	5	1.162	2.055
Between runs	6.464	5	1.293	2.286
Residual	11.310	20	0.565	
Total	42.406	35		

TABLE 5

Analysis of variance for $T_{50\%}$ values of salicylic acid tablets coated together with radiolabelled tablets

Source of variation	Sum of squares	Degrees of freedom	Mean square	F statistic
Between batches	37.826	5	7.565	4.193
Between cells	5.680	5	1.136	1.588
Between runs	7.075	5	1.542	1.170
Residual	36.071	20	1.804	
Total	86.652	35		

isotope in the anterior-posterior plane within the body. To minimise errors arising from the latter effect, both anterior and posterior images were taken once the tablet was observed to be in the lower small intestine. This enabled more accurate quantitative information on the behaviour and disintegration of the tablet to be obtained by calculating the geometric mean of the anterior and posterior counts in defined regions of interest as described by Tothill et al. (1978) and Hardy and Perkins (1985). The times for the intact tablets (or 50% of the activity ($T_{a50\%}$) of disintegrated tablets) to pass through various regions of the gastro-intestinal tract were recorded. The small intestinal transit (SIT) time was calculated as the difference between the time (either of the intact tablet or $T_{a50\%}$) through the ileo-caecal junction (ICJ) and the gastric emptying time. The time in the ascending colon was taken as the difference between arrival at the hepatic flexure and time of passage through the ICJ. The position of the majority of the activity (> 50%) after 12 h was also recorded. The results are summarised in Table 6.

The mean gastric emptying time of the tablets was 2.4 ± 1.7 h, which is in accordance with literature values for relatively large (10 mm diameter) non-disintegrating tablets on a fasted stomach.

The time during which the tablets remained in the lower small intestine was recorded as, in this position, the pH in the gastro-intestinal tract has been observed to be at its maximum (normally greater than 7) (Evans et al., 1988). The time the dosage forms remained in the lower small intestine, the time at which they passed through the ICJ and consequently the SIT times were very variable. Both the mean SIT time of 6.5 ± 3.8 h (taking the minimum time in cases where the actual time was undetermined) and its variability (between 2.9 and > 13 h) were much greater than the normally accepted values found by Davis et al. (1986) in 201 studies with a variety of dosage forms. The much shorter SIT times quoted previously are possibly a consequence of the termination of many of the studies when the ICJ had been reached and not when the dosage form had entered the colon. True SIT times can only be determined using gamma scintigraphy, when transit is followed into the colon. Other studies have noted lag times of various duration at the ICJ, particularly for single units. Fallinborg et al. (1989) observed that radiotelemetry capsules (7 mm in diameter, 24 mm in length) were located in the distal ileum for 65% of the SIT time. They obtained SIT times ranging from 2.8 to more than 14 h with a median value of 8 h ($n = 39$). Marlova et al. (1987) reported periods of stasis at the ICJ for non-disintegrating matrices ranging from 2 to 20 h.

Once the intact tablets entered the colon, they traversed the ascending colon relatively quickly (< 2.1 h). This observed rapid colonic transit agrees with the results of Krevsky et al. (1986), but is in conflict with the theory that the ascending colon is the major site of fermentation and stagnation of colonic contents (Kerlin et al., 1983). In the colon, stagnation (> 1 h in the same place) of the major part of the dosage form was observed either at the hepatic flexure (volunteers 1, 4, 5, 7) or the splenic flexure (volunteer 3). Stag-

TABLE 6

Gastro-intestinal transit times (in h) for Eudragit S® coated tablets

Volunteer	Gastric emptying	Time in lower small intestine	Time through ileo caecal junction	Small intestinal transit time	Time in ascending colon	Main position at 12 h
1	3.0	1.8	6.1	3.1	2.1	hepatic flexure
2	1.6	> 15	> 15	> 13	–	ileo-caecal junction
3	1.4	5.5	8.7	7.3	0.5	splenic flexure
4	6.1	3.6	10.8–12.0 [a]	5.1	–	ascending colon
5	2.0	1.1	4.9	2.9	0.2	hepatic flexure
6	1.3	> 12	> 12	> 10	–	ileo-caecal junction
7	1.3	0.0	5.0–5.5 [a]	4.0	0	hepatic flexure

[a] Range as tablet disintegrated prior to ICJ.

nation of non-disintegrating tablets of similar diameter (11 mm) at the flexures has been previously observed by Price et al. (1991).

12 h after administration, the position of the tablets varied between the ICJ and the splenic flexure. Eating or defaecation during the study did not result in a significant change in position of the dosage form. This observation is in agreement with the results of Krevsky et al. (1986) who found no correlation between the number of bowel movements per day and the movement of material through the colon.

Both the disintegration times and the positions of disintegration were very variable (see Table 7 and bore no relationship to the time the dosage form passed through the ileo-caecal junction. Evidence from these studies suggests that when a tablet disintegrates in the colon, spreading of its contents occurs aborally. Fig. 1, which was obtained by constructing regions of interest around the tablet and its distally released contents illustrates this effect for volunteer 1. The geometric means of the radioactive counts in these regions were used to produce values for the percentage of activity remaining in the dosage form and the percentage of released activity at a particular time. The summation of these two percentages accounted for the total activity. This spreading of released radiolabel did not occur rapidly in all volunteers.

The images taken the following morning showed the presence of radiolabel distributed throughout various segments of the colon but particularly concentrated at the flexures and in

TABLE 7

Times and positions of disintegration of radiolabelled tablets

Volunteer	Disintegration	
	Time (h) after administration	Position
1	8.2	hepatic flexure
2	> 15	not determined
3	12.0	splenic flexure
4	9.3	terminal ileum
5	12–24	hepatic flexure
6	12–24	ICJ
7	5.0	terminal ileu

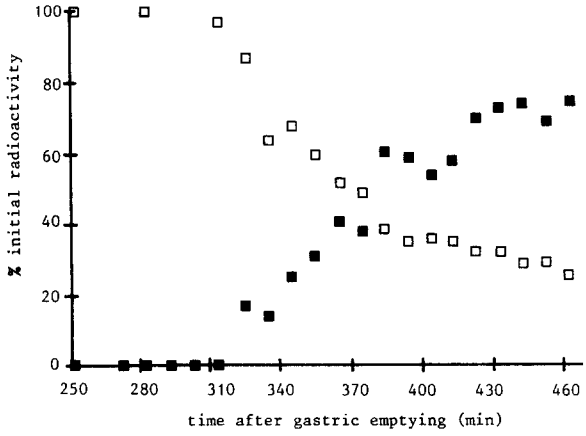

Fig. 1. Graph showing the percentage of radioactivity remaining in the tablet (□) and the percentage of radioactivity released distally (■) from the tablet plotted against the time after gastric emptying (volunteer 1).

the rectum. This distribution after 24 h suggests that the released radiolabel, which was most likely to be in a liquid form, traversed the colon more slowly than the intact tablet. This could be an exaggerated form of colonic sieving, which has been observed previously in several studies (Davis et al., 1984; Hardy et al., 1985).

The presence of non-disintegrated tablets at the ICJ for many hours for volunteers 2 and 6 suggested that the pH in this region was below 7. Fallinborg et al. (1989) found that in 17 of the 39 healthy volunteers who participated in their study, the pH began to drop, by 0.1–0.8 of a pH unit, during the last few hours of SIT, before it fell more sharply as the radiotelemetry capsule entered the caecum. No similar drop in the pH of the terminal ileum was reported in the study of Evans et al. (1988), but this may have been due to the detection method. A lower pH in the terminal ileum could be due to overgrowth of bacteria into the ileum. Studies on normal ileal contents in healthy subjects have shown high counts of many faecal bacteria (Hamilton et al., 1970). Because of the fall rather than rise in pH at the trigger region, the polymer coat will need to start to dissolve prior to colonic entry as it is improbable that the pH will rise above 7, if at all, until the distal parts of the colon are reached (Evans et al., 1988; Fallinborg et al., 1989).

Premature disintegration will result in either

degradation of a labile drug or absorption of a drug intended for local action. If disintegration occurs late, much of the opportunity for absorption or local action in the colon will be lost, particularly if there is no back mixing of contents. It is interesting to note that none of the tablets disintegrated in the caecum or ascending colon, the regions of lowest pH in the intestines; they had either disintegrated prior to reaching these regions of low pH or later, when the pH would be expected to have risen again.

In summary, this investigation, in accordance with previous in vitro results (Ashford et al., 1992), demonstrates that although a Eudragit S® coating is capable of protecting a tablet during its passage through the stomach and upper small intestine, its site specificity is poor. The long lag times at the ICJ and quicker transit indicate that a single unit may not be the best dosage form for a colonic drug delivery system. The late disintegration of a single unit dosage form poses a particular problem due to the aboral release of contents, and will result in loss of much of the opportunity for local action or absorption in the proximal colon.

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