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Does the site of intestinal delivery of oleic acid alter the ileal brake response?

Clair L. Dobson [a], Stanley S. Davis [a], Sushil Chauhan [b], Robert A. Sparrow [c], Ian R. Wilding [d,*]

[a] *School of Pharmaceutical Sciences, Nottingham University, Nottingham NG7 2RD, UK*
[b] *SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex CM19 5AD, UK*
[c] *School of Biomedical Sciences, Queens' Medical Centre, Nottingham NG7 2UH, UK*
[d] *Pharmaceutical Profiles Ltd, 2 Faraday Building, Highfields Science Park, Nottingham NG7 2QP, UK*

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Abstract

Previous work has demonstrated that high doses of oleic acid can activate the ileal brake but the importance of site of delivery has yet to be investigated. The objective of this study was to use modified release capsules to release oleic acid in different regions of the intestine. When tested by in vitro dissolution in pH 6.8 phosphate buffer, one batch released the contents almost immediately, another after around 30 min and the last batch after around 60–70 min. The effect of oleic acid release site on the ileal brake was assessed by the measurement of transit time of radiolabelled non disintegrating tablets by γ scintigraphy. The results demonstrated that the transit of tablets could be slowed down by oleic acid and therefore it appears the ileal brake can be activated along the entirety of the small intestine.

Keywords: Ileal brake; Oleic acid; Tablets; Gastrointestinal transit; Scintigraphy

1. Introduction

The ileal brake is a feedback mechanism regulating movement in the small intestine. It was originally considered as a slowing of bulk transit in response to the presence of fat in the ileum (Spiller et al., 1984). This meant that if dietary fat was not digested/absorbed higher up the intestine, the brake mechanism would ensure that the transit of the undigested fat through the rest of the ileum was slowed down and hence a longer time made available for completion of digestion. However, fats are not the only substances that have been found to activate the brake. Certain substances, which are lipid-soluble, e.g. lecithin also slow down transit, as do bile acids (Brown et al., 1990). Thus, it appears that the ileal brake is not totally substrate specific. Moreover the mechanism is not solely dependent on lipid solubility

* Corresponding author. Tel.: +44-115-9436565; fax: +44-115-9225321.
E-mail address: iwilding@pharmprofiles.co.uk (I.R. Wilding)

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since some lipid-soluble compounds, e.g. oleyl alcohol do not activate the brake (Brown et al., 1990). In addition undigested triglycerides have been shown to have no effect on transit time (Spiller et al., 1984). From this information, it has been deduced that partial digestion of triglycerides is needed, which supports the idea that the mechanism is designed to aid the completion of dietary fat digestion. Other dietary components have also been shown to trigger the ileal brake, e.g. partially digested protein and starch (Spiller et al., 1988). However, apparently a large dose is needed to produce an effect on transit and because this involved ileal infusion of large volumes it proved too uncomfortable for the volunteers. This observation further suggests that calorific load may be an important stimulus of the ileal brake, since fat contains the highest amount of calories per unit weight.

Fatty acids have been used as potential brake activators in many published studies, both singularly and in mixtures, e.g. lipid emulsions. Oleic acid has been used most commonly (Spiller et al., 1988; Pironi et al., 1993; Dreznik et al., 1994). It was chosen as the substance tested in our initial study to observe whether the ileal brake could affect tablet movement through the gut (Dobson et al., 1999). Previously published studies had not investigated the effect of the ileal brake on the transit of pharmaceuticals. These studies concentrated on following the transit of a test meal and seeing if this was altered by the chosen stimulus (Pironi et al., 1993). If it is possible to slow down tablet transit via the ileal brake, using a method similar to that used to slow down food, then there exists a significant commercial opportunity for products containing drugs exclusively absorbed in the small intestine. If the drug could be formulated with an excipient, at a pharmaceutically relevant concentration, which would activate the ileal brake, then residence time in the small intestine may be increased, potentially leading to an increase in drug bioavailability.

Initial feasibility research showed that the transit of tablets could be slowed down by stimulation of the ileal brake (Dobson et al., 1999). This study used two sets of non-disintegrating placebo radiolabelled tablets as a 'model' pharmaceutical system and the progress of the tablets through the small intestine was monitored using γ-scintigraphy. Different doses of oleic acid were tested as a brake activator and delivered to the ileum using a modified release capsule (Watts, 1995). The general trend, seen in most volunteers, was that the small intestinal transit time (SITT) of the tablets was extended as the dose of oleic acid increased. The effect was dose-dependent and was greatest when the tablets were administered simultaneously with the capsule. Although the results were promising in that a significant increase in SITT was shown to occur, the effect was not observed in every volunteer. However although the capsules were radiolabelled it was not possible from the image analysis, to determine the site of release, due to interference from the ^{99m}Tc labelled tablets. Therefore, it is possible that the oleic acid was not actually released at the optimum site of action, thus, causing no effect. In addition although in vitro dissolution testing suggested that release should occur in the ileum, the in vivo situation may have been different if the small intestinal transit had been quicker than anticipated. Finally it is also conceivable that the quantity of fatty acid used in the study was insignificant, when compared with the total amount of fat found in the diet, i.e. if the gastrointestinal (GI) tract is consistently being presented with large quantities of digested fat then any feedback mechanism may become desensitized.

The present study was designed to address these questions and determine whether the site of delivery of oleic acid within the intestine affected the ileal brake response. Recent work (Lin et al., 1997) has suggested that the optimal site for delivery should be the ileum. Lin et al. (1996a) showed that although most fat is absorbed from the proximal small bowel, there is also absorption in the ileum, with the amount of fat passing the mid small intestine being proportional to the quantity ingested. Exposure of the distal small intestine to chyme, caused a greater absorption of fat higher up the GI tract, due to the inhibitory effect on gastric emptying and jejunal transit. This supports the idea that the ileum is the primary site for any braking mechanism within the small intestine, al-

though the slowing of transit is not localised to this area. However a distinct braking mechanism has also been identified in the jejunum (Lin et al., 1996b,c). The activation of this mechanism is found to occur at higher concentrations of nutrients than the ileal brake and infusion of test solutions into the jejunum did not elicit as great a response as when they were infused into the ileum (Spiller et al., 1988). This increased potency of fat in the ileum rather than jejunum, may be related to the amount of fat normally reaching these areas (Lin et al., 1997). The jejunum is frequently exposed to fat whereas the ileum does not usually receive fat in such large quantities. Thus if the brake operating in the jejunum was very sensitive to luminal fat then an effect would be frequently observed, so a higher threshold value is physiologically relevant. Conversely, any significant amount of fat in the ileum, indicates a larger oral intake making it more beneficial to slow transit, and necessitating a more sensitive mechanism. It has also been suggested that at least some exposure of the ileum to the activating nutrients is necessary before the jejunal brake is stimulated (Brown et al., 1994). These observations suggest that the greatest effect on small intestinal transit time would occur when oleic acid was delivered to the ileum.

This study aimed to investigate if the ileum was the optimal site of delivery of oleic acid or if a general brake response occurred regardless of the site of delivery. Three different sites within the intestine; the duodenum, mid-small intestine and the terminal ileum, were chosen as the areas to be targeted in the present study. The targeting was achieved by coating three batches of capsules each containing 1200 mg oleic acid to give different in vitro dissolution times at pH 6.8, approximately corresponding with the time that should be taken to reach the respective points in the small intestine. As with the previous study the effect of the oleic acid on transit was measured by γ scintigraphy. The possibility that the usual diet of the volunteers would mask the response to the ileal brake was minimised, by placing them on a low fat diet, started 1 week before the study. This should have given sufficient time for the GI tract to adjust to the new diet (Price et al., 1991) and therefore should reduce any effect that the volunteers' normal diet would have on the study findings.

Thus the aim of this study was to determine whether the delivery of oleic acid (1200 mg), to different sites within the small bowel, has an effect on the small intestinal transit time of non-disintegrating tablets.

2. Methods

2.1. Volunteers

The study was undertaken using seven healthy volunteers ($n = 3$ male; $n = 4$ female). All volunteers passed a pre-study medical and met the protocol requirements. All subjects were required to follow a controlled low fat diet throughout the study period commencing 1 week prior to the first study day. The total amount of fat to be eaten on each day did not exceed 40 g. A 'normal' diet (on which there is no significant weight change) comprises about 70–90 g fat per day. The diet of each volunteer was individually determined and showed little variation each week, the average daily intake being around 30–35 g fat.

2.2. Protocol

The volunteers fasted from 10 pm overnight before each study day. They were dosed at around 8 am with the test formulation (or control) and five non-disintegrating 6 mm placebo tablets labelled with ^{111}In. The volunteers ate a controlled lunch and tea at 4 and 9 h post dose, respectively. The test formulations were: A-no capsule; B-1200 mg oleic acid capsule delivered to the duodenum; C-1200 mg oleic acid capsule delivered to the mid small intestine; D-1200 mg oleic acid capsule delivered to the terminal ileum.

2.3. Dosage forms

2.3.1. Capsules

Size 000 gelatin capsules were initially filled with 200 mg oleic acid (Thornton and Ross). The cap and body were sealed together with adhesive.

The resulting join between the parts of the capsule, was sealed to prevent leakage of liquid contents. The seal was achieved by application of a band of gelatin using a Quali-Seal (Elanco) bander. The capsules were stored upright until the gelatin hardened. The filled capsules were then coated, using a Niro Aeromatic coater, with a mixture of Eudragits® (Röhm Pharma, Darmstadt) which resist degradation in the acidic stomach, then give a time-delayed release in the small intestine (Watts, 1995). Three different batches of capsules were produced, with different average coating weight gains per capsule, corresponding to different in vitro release times at pH 6.8 and potentially different target sites of delivery within the small intestine. Dissolution testing was carried out for 2 h in hydrochloric acid pH 2 then the media was changed to phosphate buffer at pH 6.8. The time taken for dissolution in pH 6.8 was as follows: Batch B-less than 5 min, Batch C-20 min, Batch D-70 min.

The capsules were radiolabelled the day before dosing, by the injection of radiolabelled Amberlite resin IRA-410 into the capsule. The resin was radiolabelled by wetting with a solution of ^{99m}Tc pertechnetate, then dried, by heating to 80°C until constant weight, then sieved. A weight of resin, size fraction < 63 μm, labelled with the desired amount of activity, was suspended in oleic acid. A hole was drilled in the top of the capsule and the labelled suspension injected through. Further unlabelled oleic acid was added, to give a total of 1200 mg in each capsule. The hole was then sealed with adhesive. The total amount of activity in each capsule, at the time of administration, was 4 MBq.

2.3.2. Tablets

Placebo non-disintegrating tablets were prepared to contain ^{111}In. Briefly, Amberlite IRP-69 resin was labelled with ^{111}In by wetting with a solution of indium chloride and drying until constant weight. A quantity of resin to give the correct activity was added to ethylcellulose and the powder mix directly compressed into tablets using a Manesty F3 tablet machine. The resulting tablets were 6 mm in diameter, had a hardness of between 7 and 9 kgF and a weight of 100 mg. They were then coated with two coats of each of two different solutions (containing cellulose acetate or ethylcellulose) designed to prevent disintegration within any part of the GI tract. The approximate activity, at the time of administration, was 0.2 MBq per tablet.

2.4. Image acquisition

Imaging commenced immediately post dose. Before dosing, an external marker, labelled with ^{111}In, was positioned on the volunteers' abdomen, over the right lobe of the liver. Anterior images of 60 s duration were acquired approximately every 12–15 min throughout each study day until 10 h post dose. The images were stored on an optical disc.

2.5. Analysis of images

If oleic acid, and hence the ileal brake, has an effect on tablet transit, this will be demonstrated by changes in the time taken to travel through the small intestine, i.e. SITT. Therefore this parameter was used to assess any differences between the test groups.

The use of the external marker, which remained fixed for the entire study day, allowed the relative positions of the tablets and capsule to be determined for each image. By viewing consecutive images it was possible to define the different anatomical regions of the GI tract (stomach, small intestine or colon) and therefore to determine the position of the dosage forms and hence when they moved from one region to another. The times quoted for gastric emptying (GE) and colon arrival (CA) are the mid-point of the times of the images between which the events occur. Once the GE and CA values of each of the five tablets was determined the mean value of both events could be calculated. The difference between the two was taken as the SITT. In some cases CA did not occur during the imaging period, therefore the timing of the last image was taken as the minimum time and values are quoted as greater than this time. The site at which release of the capsule contents occurred could also be determined from the images.

The oesophageal transit time (OTT) is the difference between the dosing time and the time at which the capsule enters the stomach.

3. Results and discussion

Using scintigraphy it was possible to determine the site of release of oleic acid from the capsules within the gastrointestinal tract. The values of SITT, GE and CA are given for the various release sites in Table 1.

The OTT for the test capsules are shown in Table 2.

The results from the current study confirm the finding from our initial investigation (Dobson et al., 1999) that the ileal brake, once activated by oleic acid, can slow down the transit of tablets in the small intestine. A doubling of transit time was seen in some of the volunteers, when dosed with oleic acid, compared to the control, but as in the previous study, there were volunteers in which no effect was seen. One volunteer actually gave the slowest small intestine transit with the control

Table 1
Table showing GE, CA and SITT (in min) of the tablets when oleic acid was released at different sites in the small intestine

Volunteer		Oleic acid release site									
		Control	Stomach	Duodenum	Mid SI		ICJ		Colon		
1	GE	75	195		i.	55					
	CA	246	>583			>506					
	SITT	171	>388			**>451**					
	GE				ii.	69					
	CA					352					
	SITT					**283**					
3	GE	27	55			55					
	CA	225	257			243					
	SITT	198	202[a]			**188**					
4	GE	81	72				i.	68			
	CA	250	>557					362			
	SITT	169	>485					**294**			
	GE						ii.	84			
	CA							>457			
	SITT							**>373**			
5	GE	241	101		i.	34					
	CA	418	401			151					
	SITT	177	300			**117**					
	GE				ii.	31					
	CA					336					
	SITT					**305**					
6	GE	124	141		i.	79					
	CA	426	398			362					
	SITT	302	257			**283**					
	GE				ii.	42					
	CA					201					
	SITT					**159**					
7	GE	59	46			32			28		
	CA	246	250			231			250		
	SITT	187	204[a]			**199**[a]			222		
8	GE	34		107		55		102			
	CA	276		167		376		367			
	SITT	242		60		**321**		265[a]			

[a] Capsule emptied from stomach a significant amount of time after tablets.

Table 2
Table showing OTT (in min) of capsules, intended target site and actual release site of contents

Volunteer	Intended target site of release		
	B Duodenum	C Mid small-intestine	D ICJ
1 OTT	17	1	27
Actual release site	Mid small intestine	Stomach	Mid small intestine
3 OTT	86	42	21
Actual release site	Stomach[a]	Stomach	Mid small intestine
4 OTT	10	11	58
Actual release site	Stomach	ICJ	Mid small intestine
5 OTT	22	27	23
Actual release site	Stomach	Mid small intestine	Mid small intestine
6 OTT	47	42	21
Actual release site	Stomach	Mid small intestine	Mid small intestine
7 OTT	22	22	19
Actual release site	Stomach	Mid small intestine	Colon
8 OTT	0	32	7
Actual release site	Duodenum	Mid small intestine	ICJ

[a] Contents were almost fully dispersed in stomach which delayed considerably GE of tablets — only one tablet reached colon during imaging period therefore no SITT given in Table 1.

(SITT, 302 min) and thus it would appear that the oleic acid speeded up the transit (SITT, 257, 283, 159 min). As the capsules were radiolabelled, it was possible to determine from the analysis the major site of release. From this it could be seen that most of the capsules which were designed to release in the duodenum, actually showed initial disintegration in the stomach. However, in most cases this was not a problem, since when GE of the capsule occurred, most of the activity also left the stomach and was therefore, presented to the duodenum. The presence of oleic acid in the stomach slowed down the GE of the tablets in some volunteers and in subject 3, with formulation B, some of the tablets did not leave the stomach at all during the study day.

Another factor which may have influenced the results is that in many cases, the capsule stuck in the oesophagus for at least 20 min and often, further water had to be swallowed before it was dislodged to the stomach. It can be seen from Table 2 that in five cases, a delay of over 40 min occurred, before the capsule reached the stomach. During this time it is possible that the mechanical action of peristaltic motion damaged the coating, hence causing early release of contents. It is probable that the sticking occurred due to the large size (000) of the capsules used, even though capsules of similar size have been used in other scintigraphic studies (Watts et al., 1992) with no reported problems. However, these capsules used in the current work were somewhat wider than standard 000 capsules due to the additional gelatin band. They were dosed after an overnight fast and only 100 ml water was consumed before swallowing the capsule, so a lack of fluid to lubricate the oesophagus may have also contributed to the capsule sticking. The size of capsule and lack of dosing fluid are both factors which have been implicated in the sticking of capsules in the oesophagus (Hey et al., 1982; Swisher et al., 1984).

It appears from Table 2 that the increased OTT often led to release of the capsule contents earlier than the target site, especially with those capsules targeted to the duodenum. The longest OTT (86 min), was seen in subject 3 with formulation B, the example mentioned previously where the GE of the tablets was inhibited. In four instances the lodging of the capsule in the oesophagus, actually led to the capsule leaving the stomach about 1 h after the tablets (see Table 1). In all these cases, not surprisingly, the resulting SITT values showed very little difference from control. In subject 7,

this problem occurred with formulations B and C which had SITT of only 17 and 12 min, respectively, longer than the control. If the problem with these four cases is considered, it becomes apparent from Table 1 that nine of the 16 remaining SITT values show an increase from the control. This is essentially the true extent of the brake action as it only considers the SITT values where the oleic acid is delivered to the small intestine before or along with the tablets. Hence, an ileal brake activation is seen in over half of the cases.

Surprisingly it appears there is no single release site giving a more consistent or larger increase in SITT. The fact that only three of the capsules were released at the ICJ, means that it is not possible to draw a definitive conclusion from these results. There is not a significant difference in the results obtained from the capsules that released their contents in the stomach (then emptying into the small intestine) and those that released their contents in the mid-small intestine. The lack of difference between the results, applies to both the number of times an effect was observed and also the magnitude of the effect. This suggests that activation of the ileal brake mechanism is perhaps not localised to the ileum, but can occur through the small intestine as a whole, including the duodenum and ICJ.

It follows that the ileal brake is not a feedback mechanism which acts only to correct a problem occurring with digestion when lipid products are seen in the distal small bowel. If this were true, then activation of the ileal brake would only occur in that part of the gut. Since the mechanism can be activated in the proximal small intestine, this implies that the process acts as an aid to digestion by immediately slowing down transit when activating substances are first detected. There is no requirement for such substances to reach the terminal small bowel before activation occurs. This is a reasonable conclusion, since it is known that GE can be inhibited by the presence of fat in the duodenum (Spiller, 1990), so the same stimulus may also activate another feedback mechanism, namely the ileal brake.

Another finding from this study is that there does not appear to be any increased effect seen by placing the volunteers on a low fat diet. The results are comparable to those obtained in a study (Dobson et al., 1999) where the volunteers' diets were not controlled in any way and may question the relevance of continuing to use a low fat diet in future studies.

The conclusion from this study is that the ileal brake mechanism can be activated along the length of the small intestine. Thus, it appears that an increase in brake response is not achievable by selective targeting in the small bowel and is likely to occur only through using different brake activating agents.

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