The Effect of Oleic Acid on the Human Ileal Brake and Its Implications for Small Intestinal Transit of Tablet Formulations

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Purpose. A human volunteer study was carried out to investigate whether activation of the ileal brake mechanism affects the transit of tablets through the small intestine.

Methods. Oleic acid, which has previously been shown to activate the brake, was delivered to the small intestine in a modified release capsule at doses of 300 mg, 600 mg and 1200 mg. The effect of the oleic acid was determined by measuring the transit of two sets of radiolabelled tablets by gamma scintigraphy. One set of tablets was dosed with the capsule and the other one hour later.

Results. The results show that in the majority of the volunteers small intestinal residence time was greater with the oleic acid than control. The effect was most pronounced in the tablets given concomitantly with the capsule and with the higher doses of oleic acid.

Conclusions. The ileal brake, activated by oleic acid, can slow the transit of tablets through the small intestine.

KEY WORDS: ileal brake; oleic acid; tablets; gastrointestinal transit; scintigraphy.

INTRODUCTION

The ileal brake is a physiological feedback mechanism, which acts to regulate 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 (1). As fat is mainly absorbed in the jejunum any such material that reaches the ileum can be considered to have passed the preferred absorption site in the gut. This mechanism, once activated by food in the ileum, would therefore slow the transit of the remaining fat through the upper small intestine allowing further digestion and absorption to take place. Although fats are the main compounds which have been investigated in this regard (2–6), the ileal brake has also been shown to act after ileal infusion of other substances e.g., partially digested proteins, starch, lecithin, bile acids (7). Fatty acids have been used as brake activators in many published studies, and oleic acid has demonstrated an effect in both animal and human intubation studies (3,8).

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Despite the widespread perceived knowledge of it's role in digestion the ileal brake has not been significantly investigated in man and there is no general agreement on the mechanism of action. Previous published studies have not studied the effect of the ileal brake on the transit of pharmaceutical products. The transit of oral dosage forms through the small intestine could be significantly affected by the ileal brake and a potential application for this effect would be to increase the residence time of a drug delivery system in the small bowel. This would be advantageous for those drugs whose major site of absorption is the ileum since an increase in residence time would mean a longer time for drug uptake to occur; possibly leading to an increase in drug bioavailability. This present study was designed to determine whether the ileal brake affects the small intestinal transit of a model pharmaceutical system in the form of non-disintegrating tablet preparations.

Previous studies have used various methods to measure the effect of a chosen stimulus on gastrointestinal (GI) transit time e.g., manometry (9), hydrogen breath test (4). The method chosen in this study was gamma scintigraphy (10). Radiolabelled tablets were used to assess transit and passage through the small intestine (11). The technique of gamma scintigraphy is non-invasive which allows the study to be carried out in healthy volunteers and causes no alterations in transit.

Various doses of oleic acid were chosen for testing as ileal brake activators. As stated previously oleic acid has been shown to activate the brake in both human and animal studies (3,8). The ileal brake has been shown to act in response to local stimuli in the ileum (12). Therefore, it was considered necessary to deliver oleic acid directly to the distal small intestine to elicit a response. Previous studies have achieved this via local access or intubation (8,9). Local access is only feasible in animal studies and in human volunteers who have undergone certain forms of intestinal surgery e.g., ileostomy (8). Intubation can be carried out in healthy volunteers but the procedure itself can alter gut motility (13) which is obviously not desirable in a study designed to measure a transit effect. To avoid these problems an orally dosed modified release capsule was used. The delivery system containing the oleic acid was coated with a polymer mixture which was designed to resist degradation in the acidic stomach but provide release in the higher pH of the small intestine. The *in vitro* release characteristics suggested release after 60–70 min in the small intestine. Thus oleic acid could be delivered orally and still reach the distal small intestine before it was available to elicit a response.

The aims of this study were therefore to determine whether oleic acid could affect the transit of two sets of tablets through the small intestine. One set were administered concomitantly with the oleic acid capsule and the other set one hour later. The use of two different radiolabels allowed distinction between the two sets of tablets and meant that even if the action of the oleic acid on the ileal brake was not immediate the effect could hopefully be seen with the second set of tablets.

METHODS

Volunteers

The study was undertaken in eight healthy non-smoking subjects (3 males and 5 females) who had undergone a medical

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to ensure they met the protocol requirements. The volunteers were not taking any regular prescription medication and for the 48 hours preceding each study day they were not allowed to take any medication or consume any alcohol. The protocol was approved by the University of Nottingham Medical School Ethics Committee.

Protocol

The volunteers fasted from 10 pm overnight before each study day. Over the four study days they each received the following test formulations; A—no capsule (control), B—1 × 300 mg oleic acid capsule, C—1 × 600 mg oleic acid capsule, D—2 × 600 mg oleic acid capsules. On each study day the dosing was as follows; initial dosing with test formulation and five non-disintegrating 6mm tablets labelled with ^{111}In, then one hour later five non-disintegrating 6mm tablets labelled with ^{99m}Tc. The volunteers ate a standard lunch four hours post dose.

Dosage Forms

Tablets

Placebo non-disintegrating tablets were prepared to contain either ^{99m}Tc or ^{111}In as described in detail previously (11). Briefly Amberlite resins IRA-410 and IRP-69 were radiolabelled by wetting with solutions of ^{99m}Tc pertechnetate or ^{111}In chloride, respectively, and were then dried by heating at 60°C. A quantity of resin to give the correct activity was added to ethylcellulose and the mixture compressed to form 6mm diameter tablets of weight 90–110 mg. The tablets were then coated to prevent disintegration in the gastrointestinal tract; ethylcellulose 4.5 g, acetylbutylcitrate 0.5 g, isopropanol 95.0 g followed by cellulose acetate 10 g, methanol 18 g, dichloromethane 72 g. Five of the tablets were tested for disintegration in a stirred beaker for 24 hours in pH 6.8 phosphate buffer, the coating remaining intact and no radioactivity was released into the dissolution media. The approximate activities per tablet at the time of dosing were 0.2 MBq or 0.6 MBq for ^{111}In or ^{99m}Tc, respectively.

Capsules

Starch capsules (Capill®) were filled with 200 mg oleic acid and then the cap and body sealed together with cyanoacrylate adhesive. The capsules were coated, using a Niro Aeromatic coater, with a solution containing the enteric polymers Eudragit L100 and S100 (Röhm Pharma, Darmstadt) (14). Once coated the capsule withstood disintegration in acidic media and *in vitro* release commenced after 67.5 ± 6 (mean ± SD) minutes when placed in a media at pH 6.8. The capsules were radiolabelled the day before dosing by the injection of ^{99m}Tc labelled Amberlite IRA-410 resin suspended in oleic acid. A weight of resin containing activity to give the required dose was suspended in oleic acid and injected through a hole drilled in the top of the capsule. Further unlabelled oleic acid was then added, to give a total of 300 or 600 mg in each capsule. The hole was then sealed with adhesive. The total activity, in the capsules, at the time of dosing, was approximately 1 MBq, however, in the case of formulation D this was split between two capsules i.e., 0.5 MBq in each.

Image Acquisition

Imaging commenced immediately after administration of the trial supplies. Before dosing an external marker, labelled with ^{111}In, was positioned on the volunteer's abdomen, over the right lobe of the liver. Anterior images of 60 seconds duration were acquired, by a gamma camera, approximately every 12–15 minutes throughout each study day, until nine hours post-dose. The images were stored onto an optical disc.

Analysis of Images

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 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 is the SITT (small intestinal transit time). 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.

RESULTS AND DISCUSSION

The values for mean GE and CA along with the SITT are shown for each volunteer in Table 1 and the statistical evaluation of these results is shown in Table 2. It was not possible to determine the point at which the oleic acid was released from the capsule due to the presence of the ^{99m}Tc tablets on the same images. Therefore the exact site of release within the small intestine is unknown.

The expected result from this study would be that the targeting of oleic acid, a known activator of the ileal brake, to the distal small bowel would slow transit of the tablets through the small intestine. This was in fact the effect observed in the study and although not all volunteers presented with the same pattern of results the overall trend was that at higher doses of oleic acid an increase in small bowel residence time occurred. The only results which were significant (using the Wilcoxon Rank Sum Test at 5%) were those observed with the ^{111}In tablets at 1200 mg, although the ^{99m}Tc were close to a significant difference at the 5% level at the same dose level. This suggestion that an effect is seen at 1200 mg is confirmed by consideration of the mean and standard deviation values in Table 2. However the individual SITT data does suggest that a dose-dependent response occurs with at least some volunteers (e.g., subject 2) showing effects at the lower doses. The threshold value for activation of the ileal brake appears to be in the range 300–600 mg oleic acid in most volunteers. Small intestinal transit times of tablets with the administration of 300 mg oleic acid generally showed little difference from the control but the times seen with the 600 mg dose of oleic acid showed larger differences in a greater number of volunteers. These results correlate well with a previously published intubation study (8) in which an ileal infusion containing 480 mg oleic acid demonstrated a

Table 1. Mean (Minutes Post-Dose) Gastric Emptying (GE) and Colon Arrival (CA) Times of Tablets Dosed with Oleic Acid

Volunteer		Control		300 mg oleic acid		600 mg oleic acid		1200 mg oleic acid	
		^{111}In	^{99m}Tc	^{111}In	^{99m}Tc	^{111}In	^{99m}Tc	^{111}In	^{99m}Tc
1	GE	12	22	8	37	2	37	23	109
	CA	212	184	205	176	246	213	290	231
2	GE	31	38	83	25	51	24	55	39
	CA	247	183	292	214	335	293	>487	>463
3	GE	41	32	34	66	32	90	22	42
	CA	242	191	248	189	233	308	236	196
4	GE	92	35	119	26	25	65	9	158
	CA	245	193	254	183	252	190	295	321
5	GE	77	34	21	2	81	29	22	26
	CA	244	181	273	258	335	211	>429	353
6	GE	34	76	34	27	23	188	235	186
	CA	348	301	289	262	289	288	496	431
7	GE	92	169	37	77	54	171	138	139
	CA	354	291	348	300	311	306	443	379
8	GE	21	33	149	120	19	24	50	25
	CA	243	271	314	265	269	208	>406	341

significant effect on the brake mechanism. Interestingly the present findings also showed that the ileal brake could affect the transit of tablets given one hour after the brake activator, as well as when they are administered at the same time. However, the effect was decreased in magnitude for the second set of tablets and showed in fewer volunteers; four from eight showing a response (an increase in SITT greater than one hour) with the highest dose when compared with five from eight with the first set of tablets. Also any responses obtained after administration of 300 mg and 600 mg oleic acid were less frequently observed with the ^{99m}Tc tablets.

Another conclusion from the study is the ileal brake appears not to be an all or nothing action. This type of action would occur if the ileo-caecal junction (ICJ) closed in response to brake activation and all of the tablets then remained in the ileum until the period of brake stimulation ended. However, the scintigraphic evaluation of transit of the individual tablets indicates that this was not the case. Some of the tablets passed through to the colon whilst others remained for longer in the small intestine, at the ICJ. This observation, reinforced by the fact that a greater effect is seen for the first set of tablets, suggests that the ileal brake causes a general slowing of transit throughout the small intestine.

Although the results obtained were meaningful, the effects were not seen in all of the volunteers. A number of possible explanations can be proposed. Firstly inter-subject variation may mask the effect when the dose of oleic acid was too low to stimulate an effect in some volunteers if a threshold value exists (15). However this seems unlikely since the doses are quite consistent with those having had an effect in other published studies (8). It is more likely that the dose of oleic acid was sufficient but it was not delivered to a correct site within the ileum. If the action of the ileal brake was site-specific and was localised to a certain section of the small intestine it follows

Table 2. Statistical Comparison of SITT (Minutes) with Control and Different Doses of Oleic Acid

Volunteer	Control		300 mg		600 mg		1200 mg	
	^{111}In	^{99m}Tc	^{111}In	^{99m}Tc	^{111}In	^{99m}Tc	^{111}In	^{99m}Tc
1	200	162	197	139	244	176	267	122
2	216	145	209	189	284	269	>432	>424
3	201	159	214	123	201	218	214	154
4	153	158	135	157	227	125	286	163
5	167	147	252	256	254	182	>407	327
6	314	225	255	235	266	100	261	245
7	262	122	311	223	257	135	305	240
8	222	238	165	145	250	184	>356	316
Mean	217	170	217	183	248	173	>316	249
SD	51	40	55	50	25	54	75	102
Significance (Wilcoxon's rank sum test)	—	—	NS	NS	NS	NS	S	NS

Note: S = significant at 5% level; NS = not significant.

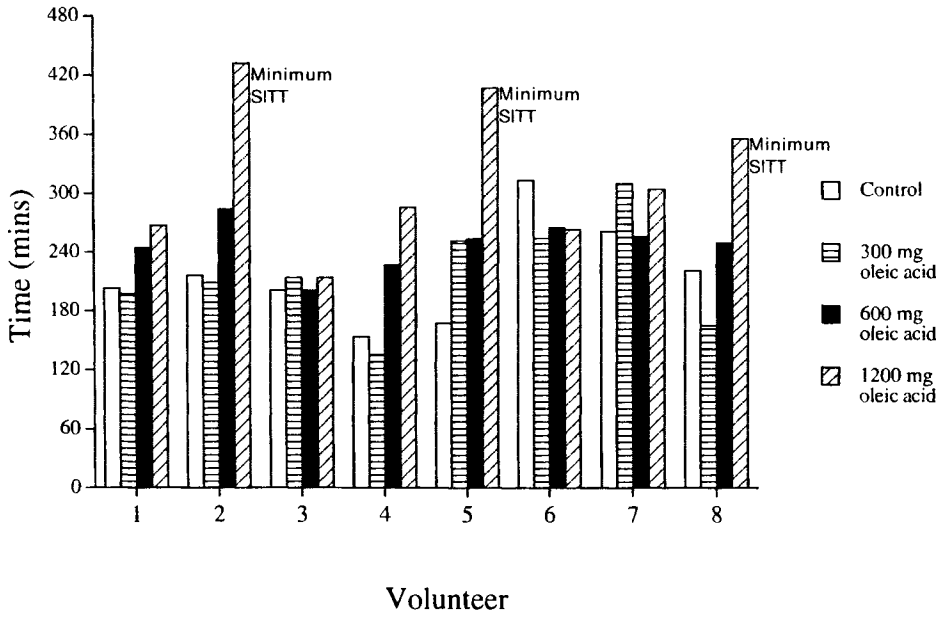

Fig. 1. Small intestinal transit time (minutes) for ^{111}In tablets when given with various doses of oleic acid. Minimum SITT indicates that the study day finished before tablets had entered colon (volunteers 2, 5, and 8 with 1200 mg).

that if the oleic acid was not released from the capsule at that point no response would be observed. It has previously been suggested that ileal delivery of fats gives a larger brake response than jejunal delivery (16). From the analysis of scintigraphic images it was not possible to determine exactly the area at which the capsule released the oleic acid due to the presence of the ^{99m}Tc labelled tablets on the images. It was possible to determine the GE of the capsule and this showed that in all but one case (volunteer 7 with 300 mg oleic acid) the capsule left the stomach during the same time period as the ^{111}In labelled tablets. If, as the *in vitro* data suggests, oleic acid release occurred after around one hour in the higher pH of the small intestine, then taking the volunteers control SITT as an average value the predicted site of initial disintegration would be mid-small intestine. Thus this suggests oleic acid would be delivered along the mid to distal small intestine. However this could not be confirmed *in vivo* and if the optimal site of delivery was not reached then any effect would be minimal. Therefore, the site of delivery may not have been optimal for action and the same dose of oleic acid delivered to a different site may have had an effect.

Another possible reason for the variation in results is the effect of the normal dietary fat intake of the volunteers. If a subject usually consumes a large quantity of lipids, which are digested to give free fatty acids, in their diet then the smaller quantity given in this study may not have been sufficient to elicit a response. This may occur due to 'desensitization' of the GI tract by constant overloading with fat.

The conclusion from this study is that intestinal transit of tablets can be slowed by the ileal brake, once activated by oleic acid. This could have possible advantages for increasing bioavailability for certain drugs which are predominately absorbed in the small intestine. Future work into the ileal brake is planned to investigate whether the factors mentioned above, site of delivery and dietary fat, play an important part in the mechanism.

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