Regional Differences in Quinine Absorption from the Undisturbed Human Colon Assessed Using a Timed Release Delivery System

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Purpose. To investigate the regional absorption characteristics of the distal gut using two markers of permeability, quinine (a transcellular probe) and ^{51}CrEDTA (a paracellular probe).

Methods. The permeability markers were delivered to the undisturbed gastrointestinal tract in 39 healthy volunteers using an oral timed-release delivery vehicle which allowed pulsed release within a particular site of the gut. Site of release was identified using gamma scintigraphy. Absorption of quinine and ^{51}CrEDTA was assessed by measuring the percent excretion in the urine using HPLC and gamma counting respectively. Serial plasma samples allowed time-concentration curves for quinine to be plotted.

Results. There was a significant trend for diminished absorption with more distal delivery of the transcellular probe, quinine, which was: 6.26 ± 0.87% (small intestine, n = 10); 4.65 ± 0.93% (ascending colon, n = 16); and 2.59 ± 0.52% (transverse colon, n = 10) of the ingested dose excreted respectively ($p < 0.001$). No such gradient was seen with the paracellular marker, ^{51}CrEDTA.

Conclusions. These results suggest that delayed release formuations shoud aim for release in the distal small bowel and proximal colon if absorption is to be miximised. Absorption by the transcellular route diminishes in the more distal colon, a fact which has implications for delayed or sustained release formulations.

KEY WORDS: transcellular; paracellular; absorption; gastrointestinal.

INTRODUCTION

It has long been the aim of the pharmaceutical industry to achieve delayed absorption of drug to treat nocturnal and early morning exacerbations of disease, e.g., asthma, rheumatoid disease, and cardiovascular disease. Such formulations taken at bedtime are inevitably situated in the distal small bowel or proximal colon by the early hours of the morning. Knowledge of the effect of site of release on absorption characteristics in the undisturbed colon is therefore highly relevant in designing such preparations.

Previous studies using a cleansed colon have suggested little intrinsic difference in absorption between proximal and distal sites (1,2), but in normal clinical use colonic contents may have an important modifying effect. We therefore aimed to study absorption by measuring the permeation of probe marker molecules from different regions of the distal gut, using a delivery system, the Pulsincap™ (3), which allowed selective delivery to various regions of the undisturbed gut.

METHODS

Subjects

Thirty-nine healthy volunteers (23 male; 16 females), age range 20–40, were recruited into the study. All subjects were free from gastrointestinal disease, and were not taking any laxatives or drugs known to affect gut motility. All were asked to refrain from excess alcohol, curries, and aspirin or non-steroidal antiinflammatory drugs during the course of the study. Females were required to have a negative pregnancy test on the morning of the study day. The study was approved by Nottingham University Ethical Committee and the Association of Radioactive Substances Advisory Committee at the Department of Health.

Study Protocol

Phase 1

In the initial phase of the study, 11 healthy volunteers (6 males; 5 females; age range 20–29) were recruited into a two way crossover study in which they were dosed with either a 5 hour (part A) or 15 hour (part B) release delivery capsule in an attempt to selectively target permeability probes to the proximal colon or distal colon respectively. There was a 2 week washout period, and the volunteers were required to adhere to a 20 gm fibre diet for 2 days before each of the study days. In part A, subjects ingested the 5 hour release delivery system at 0800 h following an overnight fast, and then consumed a standard 200 kcal breakfast of toast, butter and jam once the capsule was seen to empty from the stomach. A standard 600 kcal lunch and 1000 kcal meal were provided at 1300 h and 1800 h respectively. Blood samples were taken prior to, and at 30 minute intervals after the predicted release time (1300 h), and urine was collected in the 3 hour period leading up to the expected release time, and for the 0–20 hour period following the expected release time. In part B, subjects were dosed at 2200 h on the evening prior to the study day, so that the predicted time of release would be the same as in part A (i.e., 1300 hrs). An identical meal pattern, blood and urine sampling protocol was followed.

Phase 2

This comprised 12 healthy volunteers who were dosed on a separate occasion with a 5 hr release Pulsincap™, and 16 healthy volunteers who were dosed with a 6 hr release Pulsincap™. These 28 healthy volunteers (17M; 11F, age range 20–40) followed an identical protocol to phase 1 part A.

Pulsincap™ Delivery Capsule

The Pulsincap™ delivery capsule consists of a water insoluble body with a hydrogel plug inserted at its open end, which

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swells on contact with water and gradually propels itself from the body, thereby allowing the contents to be released. The time of release of the plug is dependent on its dimensions. Each delivery system in this study contained 50 mg of quinine hydrochloride, 1.8MBq CrEDTA dried onto sucrose, 1MBq Indium-labelled amberlite resin as an imaging agent, and an excipient. A 50 mg dose of quinine was chosen as this was sufficient to be easily detected given the highly sensitive HPLC method employed, and low enough as to be unlikely to cause any significant side-effects. The usual clinical dose for treatment of muscular cramps is 300 mg once daily and for the treatment of malaria 600 mg every 8 hours, but these doses may be associated with significant side-effects such as nausea, tinnitus, and cardiotoxicity.

Scintigraphic Imaging

Scintigraphic images were obtained using a GEC Maxi-camera set with a 20% window for simultaneous acquisition of the 140-keV radiation peak of Tc and the 247-keV radiation peak of In. Anterior and posterior images of 30 seconds duration were taken every 30 minutes after dosing. All images were stored for later analysis using a dedicated computer. Alignment of serial images was facilitated by taping small radiolabelled markers (0.2 MBq of Tc) anteriorly and posteriorly over the hepatic area. The position within the gastrointestinal tract, and the time of release of markers from the delivery system was determined by visual inspection of the serial images. Release could easily be inferred from the serial images as the area of the hot spot increased and its intensity concomitantly decreased.

Quantification of Marker Probes

The analysis of quinine was carried out using an established reversed-phase HPLC method (4). The HPLC apparatus (HP 1050) was fitted with an auto sampler and a fluorescence detector. For the assay of quinine optimum settings were: excitation = 350 nm, and emission wavelength = 450 nm. The mobile phase consisted of an acetonitrile-aqueous phosphate buffer (10 mM) mixture (70/30 v:v), containing 3 mM tetrabutylammonium bromide (TBA) and 20 mM sodium dodecyl sulphate (SDS), pH 2.5. The stationary phase consisted of a Hypersil C-18 column (5 mm) 150 × 3.2 mm protected by a guard column 30 × 3.2 mm (Phenomenex).

A protein precipitation technique was employed in the preparation of the urine samples. To 200 µl of sample, methanol (400 µl) was added, the mixture vortexed and then centrifuged at 1800 g for 15 minutes to remove the precipitate. The supernatant was transferred to a siliconised glass vial prior to injection from autosampler. Sample injection volume was 10 µl and flow rate was 0.5 µl/min. Chromatographic separations were performed at room temperature. The inter- and intra- assay coefficients of variation were found to be less than 4%. The lowest limit of detection for quinine in plasma was 3.5 ng/ml.

A 10 ml sample of urine was counted in a gamma-counter (LKB Wallac 1280) for determination of Cr-EDTA content. Reference standard solutions of Cr-EDTA were prepared at the beginning of the trials for the calculation of decay corrections. After correcting for the total volume of urine in each time interval, the results were expressed as the % of administered dose excreted.

Table 1. Number, Release Time, and Site of Release of the 5 and 6 Hour Pulsincap Delivery Systems

	5hr Pulsincap	6hr Pulsincap
Number	23	16
Release	5.5 ± 0.2 h	7.3 ± 0.3 h
Site	SI 5	SI 5
	AC 11	AC 5
	TC 4	TC 6

Note: SI = small intestine; AC = ascending colon; TC = transverse colon.

Statistics

Permeation of the marker probes from the different sites was analysed using one way analysis of variance (ANOVA), and Jonckheere's test for ordered alternatives.

RESULTS

The site and times of delivery are summarised in Table 1 (5 and 6 hr delivery systems) and Table 2 (15 hr delivery systems).

In 20 out of the 23 subjects ingesting the 5 hr release system, scintigraphic release occurred in the distal small bowel or colon. In one subject, the delivery system lay in the rectosigmoid colon and did not appear to release, and in two subjects the delivery system was retained in the stomach and therefore released in this region. Release occurred successfully in the distal small bowel or colon in all of the 16 subjects ingesting the 6 hr release delivery system.

In the 11 studies involving the 15 hr release delivery system, scintigraphic release was only observed in 2 subjects (in the ascending and transverse colons at 19.4 and 20.5 hours after ingestion). At the predicted release time, 9 of the 11 delivery systems resided in the colon (2 had been excreted). The majority of the systems, however, were located in the proximal colon (rather than the intended distal colon).

Since release of probe marker was by and large unsuccessful using the 15 hr release delivery systems, we have restricted our analysis to the 5 and 6 hr release data. We were thus able to compare delivery of marker probes to the small intestine (n = 10), ascending colon (n = 16), and transverse colon (n = 10).

Table 2. Number, and Site at Expected Release Time of the 15 Hour Pulsincap Delivery Systems

		15hr Pulsincap
Number	11	
Release	n/a	
Site	SI	0
	AC	6
	TC	1
	DC	2
	excr	2

Note: Release time is not applicable (n/a) as only 2 were evidenced to release scintigraphically. (SI = small intestine; AC = ascending colon; TC = transverse colon).

Scintigraphic release was seen a little earlier in those individuals with delivery to the small intestine compared to the ascending and transverse colons (small intestine 5.7 ± 0.4 hrs; ascending colon 6.0 ± 0.2 hrs; transverse colon 7.3 ± 0.5 hrs), resulting in a slightly greater time for permeation to occur.

Excretion of all or a proportion of the released isotope had occurred in half of the subjects by the end of the 0–20 hr urine collection period, with approximately equal proportions in each set of individuals grouped by initial release site (small intestine 5/10; ascending colon 7/16; transverse colon 6/10).

Permeation of Marker Probes

Quinine absorption diminished with progressively more distal release of capsular contents, as assessed by the 0–20 hr urine collection: small intestinal release, 6.26 ± 0.87% of the ingested dose excreted; ascending colon release, 4.65 ± 0.93% of the ingested dose excreted; and transverse colon release, 2.59 ± 0.52% of the ingested dose excreted (Fig. 1). One way analysis of variance showed a difference between these sites, with Jonckheere's test for ordered alternatives showing a significant trend for decreased absorption with progressively more distal delivery ($p < 0.001$). Permeation of EDTA, however, showed no such gradient: small intestinal release, 1.43 ± 0.43% of the ingested dose excreted; ascending colon release, 0.60 ± 0.18% of the ingested dose excreted; and transverse colon, 1.17 ± 0.64% of the ingested dose excreted (Fig. 2).

Although time concentration profiles of plasma quinine seemed to show a trend for a more rapid upstroke with more proximal delivery (median [range] times to peak concentration: small intestine 1.9 [0. 5–3.3] hours, ascending colon 2.3 [0.6–17] hours, transverse colon 2.5 [1.0–8.0] hrs), this was not statistically significant. There was however a significant trend for greater peak concentrations with more proximal delivery (median [range] concentration: small intestine 0.66 [0.13–1.06], ascending colon 0.38 [0.02–0.61], transverse colon 0.18 [0.08–0.60], $p < 0.005$, Jonckheere's test for ordered alternatives, see Fig. 3).

DISCUSSION

The Pulsincap™ delivery system allowed us the opportunity to examine the absorption characteristics of the colon in a non-invasive physiological manner, by delivering a bolus quantity of marker probe to a selected region. A previous study had suggested that a 5 hour release time would allow successful targeting of the proximal colon (5).

The present study shows that precise targeting was not possible due to interindividual differences in gastrointestinal transit rates. Indeed, despite the reasonably precise release times obtained from the 5 and 6 hour Pulsincaps™, delivery occurred in the small intestine in almost 1/3rd of cases, and as far distally as the transverse colon in another 1/3rd. Nevertheless, selective release in the ileocaecal region (distal small bowel or ascending colon) occurred in 26/39 (67%) individuals (using timed 5 and 6 hour delivery systems. This compares with a figure of 86% in a study employing a methylacrylate ('Eudragit') coating to protect gelatin capsules against disintegration within the stomach in 14 subjects (6).

Selective delivery to the distal colon represented a more difficult challenge. The descending and sigmoid colon have thick muscular walls (7) designed primarily for propulsion, and recent studies have suggested that this region serves as a conduit, in contrast to the storage role of the more proximal colon (6,8).

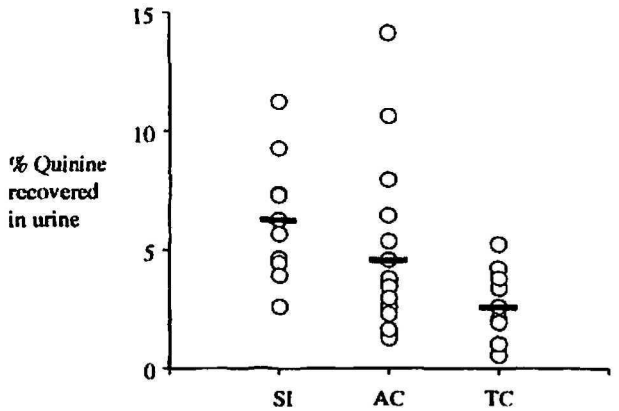

Fig. 1. Percent of quinine appearing in the urine following release in the distal small bowel (SI), ascending colon (AC), and transverse colon (TC).

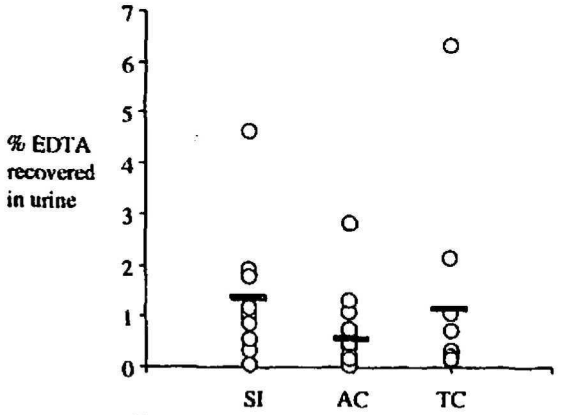

Fig. 2. Percent of ^{51}CrEDTA appearing in the urine following release in the distal small bowel (SI), ascending colon (AC), and transverse colon (TC).

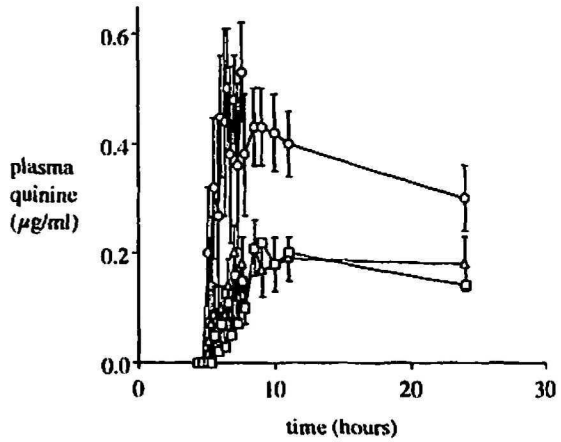

Fig. 3. Graph showing the mean concentration of quinine in plasma versus time, following small intestinal release (circles), ascending colon release (triangles), and transverse colon release (squares). Standard error bars are shown.

Thus there is likely to be a much smaller 'window' of time available for release to result in effective targeting of this region. Additionally, as residence time in the ascending colon has been shown to be approximately 12 hours (9), a substantial delay in release time would be required. *In vitro* work had shown the Pulsincap™ delivery system successfully releasing after a delay of up to 15 hours, and it was therefore this configuration which was used to effect selective delivery to the distal colon.

Our attempts to target permeability probes to the distal colon using the 15 hour delivery system were however unsuccessful on two counts. First and unexpectedly, the majority of the delivery systems were situated in the proximal colon at their predicted release time (15 hours), and no more further advanced when compared to the 5 hour systems viewed 6 hours after dosing (Fig. 4). This relative stagnation may in part be explained by the different dosing times in the two arms of the study. In the 15 hour arm, subjects were dosed at 2200 hrs so that the predicted release time (1300 hrs) would be identical to the 5 hour arm of the study, and therefore meal patterns would be the same. Thus in this arm of the study, subjects would have slept for approximately 8 of the 15 hours, and sleep has been shown to reduce colonic electrical and contractile activity (10–14). Delayed nocturnal gastric emptying (15) and reduced propagation velocity of the intestinal migrating motor complex (16) may also have been contributory, as supported by the finding that in 2 individuals the delivery system did not enter the colon until 12.5 and 13.5 hours after ingestion. Secondly, although reliable release from a 15 hour release Pulsincap™ had been demonstrated in simulated intestinal contents in vitro, there had been no previous in vivo experience with this configuration. In fact, scintigraphic release was only evident in 2 of the 11 volunteers in phase 1 of the study (although plasma samples showed evidence of release in a further 3 individuals).

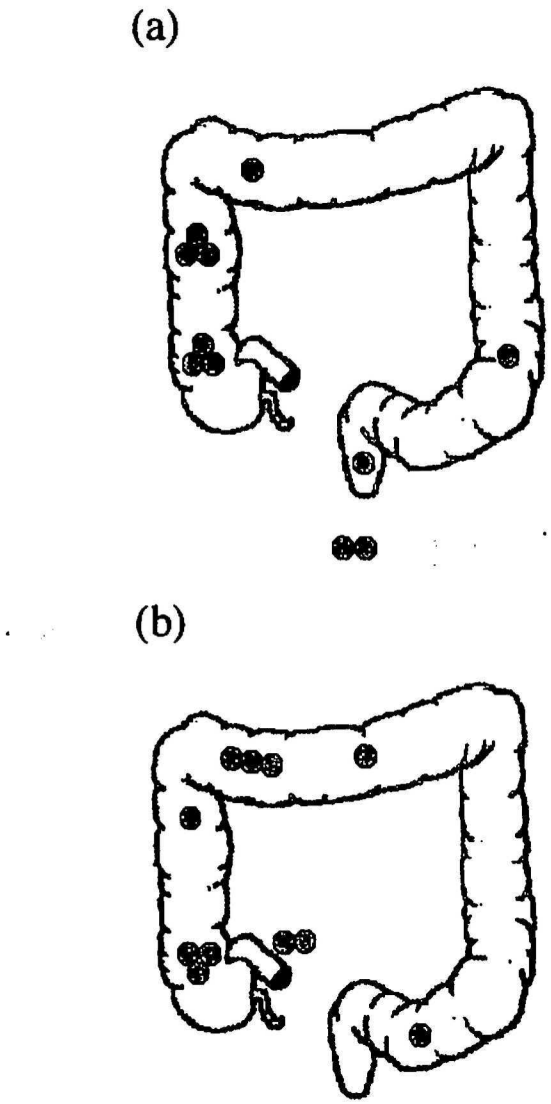

Fig. 4. Postion of (a) 15 hour timed-release delivery systems within the colon 15 hours after ingestion, and (b) 5 hour timed-release systems (or released contents) 6 hours after ingestion. The distribution is remarkably similar despite the differing lengths of time post ingestion.

Targeted delivery to the distal colon using the 15 hour delivery capsule was unsuccessful for two reasons. Firstly, the capsules were no further advanced 15 hours after ingestion than the 5 hour capsules viewed 6 hours after dosing (Fig. 4). This was probably related to the period of sleep following dosing, which is known to reduce colonic electrical and contractile activity (10–14), delay gastric emptying (15), and reduce the propagation velocity of the intestinal migrating motor complex (16). Secondly, release was only successful in 5 individuals (2 scintigraphically; 3 additionally on plasma measurements of quinine). Despite these shortcomings, we were still able to compare regional absorption characteristics of the distal gut as a result of the interindividual variations in transit of the 5 and 6 hour delivery systems, which led to a spread of initial release sites. Our results show a clear trend for reduced absorption of quinine as one moves aborally from the distal small bowel to the transverse colon. A similar gradient however was not observed for the permeation of CrEDTA.

Intestinal permeability tests investigate the unmediated diffusion across the intestinal epithelium of medium and large sized, inert, non-metabolised, water soluble molecules. There are two main pathways: transcellular and paracellular. The former are thought to be small aqueous 'pores' (<0.4–0.7 nm) of high incidence, whereas the latter are thought to be larger aqueous 'channels' (>6.5 nm) of low incidence allowing the permeation of larger molecules such as CrEDTA (17,18). Lipid soluble substances can diffuse directly through the cell surface membrane. Monosaccharides, such as L-rhamnose (molecular mass 164 Daltons; molecular diameter = 0.83 nm) and D-mannitol (molecular mass = 182 Daltons; molecular diameter = 0.67 nm) are thought to permeate largely transcellularly, whereas disaccharides, such as lactulose (molecular mass = 342 Daltons; molecular diameter = 0.95 nm) and the radioligand 51CrEDTA (molecular mass = 359 Daltons; molecular diameter = 1.05 nm) are thought to permeate paracellularly. The relative abundance of the small transcellular pores results in greater permeation of the monosaccharides (11–19% L-rhamnose excreted 5 hrs after oral dosing) compared to the disaccharides (0.3–0.4% lactulose excreted 5 hrs after oral dosing) (18,19).

The permeability markers chosen in our study were quinine hydrochloride and Cr-51 EDTA. These materials were used because of ease of detection and lack of pharmaceutical action. The absorption of orally dosed quinine salts occurs rapidly within around 2 hours, with a bioavailability of 76–88% (19,20,21). Quinine hydrochloride is very soluble in both water

and lipid (1 gram dissolves in 16 mls water, 1 ml chloroform, and in 350 mls ether). Quinine has pKa's of 4.2 and 8.8 and is therefore partially ionised in the small and large bowel, where pH ranges from 5.5 to 7.8. Since the log P of quinine is approximately 2.1, at pH 6.7 it will be equally partitioned between water and lipid. It is therefore likely to partition both through the lipid membrane and equally through small water soluble pores within the membrane (i.e., transcellularly). Its appearance within erythrocytes (20) is consistent with these concepts. Generally, it is representative of many classes of pharmacological compounds which are absorbed through the transcellular pathway. Cr-51 EDTA is a large chelate (molecular mass = 359 Daltons) carrying a small negative charge, and is thought to permeate via the paracellular pathway. It adopts a strictly extracellular distribution when given intravenously, and is therefore thought to be unable to pass through cell membranes (ie transcellularly).

There are several possible explanations for the gradient observed with quinine. Firstly, the frequency of aqueous pores may be different between cells in different parts of the gastrointestinal tract. Secondly, the mucous layer in the colon limits access to the absorptive surface, and this layer has been shown to become progressively thicker distally (22). Finally and perhaps most importantly, the luminal contents differ between the proximal and distal colon. The right side of the colon contains liquid stool which would be predicted to promote drug dispersion, diffusion and mucosal contact whereas the left side of the colon contains viscous, dehydrated stool within which drug may become sequestered, and hence unavailable for permeation.

An earlier pilot study involving 10 subjects had suggested decreased absorption of CrEDTA with more distal colonic release (23). We found no such gradient when a larger number of subjects were studied (n = 36). It is possible that the preliminary study suffered from a type 1 error, as the numbers in each region were small (small intestine 4, ascending colon 3, transverse colon 3). It is however noteworthy that several EDTA absorption values seen in the larger study were much higher than expected. The usual upper limit of absorption of CrEDTA following ingestion as a solution is around 2.6% (24), and several EDTA absorption values seen in this study far exceed this figure. One explanation of the abnormally high values seen of these results is that pulsed delivery of sucrose and CrEDTA may result in high local concentrations which alter local permeability at the site of release, and hence confound any true differences in absorption by region.

In summary, this study has shown the feasibility of selectively targeting material to the ileo-colonic region using the Pulsincap™ timed release delivery system. Selectively targeting material to the distal colon presents particular difficulties due to a combination of temporal factors, interindividual variability in transit rates, and the reservoir function of the intervening proximal colon. Using the 5 and 6 hour timed release delivery systems to target the distal gastrointestinal tract, we have shown a gradient of absorption for the transcellular probe quinine. Whether this gradient is primarily determined by differences in the water content of the stool or properties of the mucosa-mucus barrier remains to be determined.

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