Stool Water Content and Colonic Drug Absorption: Contrasting Effects of Lactulose and Codeine

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Purpose. By varying stool water content using lactulose and codeine, we investigated the influence of luminal water content on the absorption of quinine, a transcellular probe, and ^{51}Cr-EDTA, a paracellular probe, from the distal gut.

Methods. Sixteen volunteers entered a three-way cross-over trial in which absorption of probe markers from a timed-release delivery system was determined following treatment with lactulose 20 mls tds (increasing water content), or codeine 30 gms qds (decreasing water content), and compared with control untreated values. Stool water content was assessed by freeze drying stool samples. Site of release was determined by gamma scintigraphy, and absorption was measured by plasma levels and urinary recovery of the marker probes.

Results. Lactulose accelerated ascending colon transit (3.7 ± 0.8 vs 4.5 ± 1.4 hrs, $p < 0.05$), increased stool water content (75 ± 2 vs 71 ± 2%, $p < 0.01$), caused greater dispersion of released material (dispersion score 3.4 ± 0.3 vs 1.8 ± 0.2, $p < 0.01$), and enhanced absorption of the transcellular probe quinine (4.66 ± 0.78 vs 3.02 ± 0.63%, $p < 0.05$) compared to control. Conversely codeine slowed ascending colon transit (8.9 ± 1.8 hrs), reduced stool water content (61 ± 2 vs 71.2%, $p < 0.05$), and tended to diminish absorption (2.60 ± 0.77 vs 3.02 ± 0.63%, $p = 0.20$). Within the ascending colon specifically, there was a significant trend for treatments increasing luminal water content to enhance quinine absorption (medians: codeine = 1.2%, [n = 8] < control = 2.3%, [n = 5] < lactulose = 3.2%, [n = 7], $p < 0.01$). Delivery site also had an important influence on absorption, with more distal release resulting in less absorption in the control arm (medians: small intestine = 4.4% [n = 5] > ascending colon = 2.3% [n = 5] > transverse colon = 1.5% [n = 6], $p < 0.005$).

Conclusions. Lactulose accelerates transit, increases stool water content, and enhances drug absorption from the distal gut whilst codeine slows transit, decreases stool water content, and tends to diminish absorption, compared to controls. We conclude that water content may be an important determinant in colonic drug absorption.

KEY WORDS: colon; absorption; EDTA; quinine; lactulose; codeine.

INTRODUCTION

As patient compliance drives the pharmaceutical industry to produce more once-daily dosing formulations, understanding colonic drug absorption becomes more important. Most once-daily dosage formulations can be expected to reach the colon 4 to 8 hours after ingestion, implying that during 2/3rds of the 24 hour period drug bioavailability is determined by the extent of colonic absorption. Previous studies suggest important regional differences in drug absorption with more drug absorbed from the proximal than the distal colon (1,2). Variable transit through these regions with their differing absorption rates may be an important determinant of the 24 hour drug concentration profile.

Such a gradient of absorption might be explained, in part, by changes in the epithelial barrier. Electrophysiological studies show an increasing transepithelial voltage gradient and electrical resistance as one passes from the right to the left side of the colon (3). We hypothesised however, that the most important factor determining absorption was the water content of the stool. The fluid milieu of the right-sided colon, which receives approximately 1.5 L of chyme daily (4), promotes drug dispersion and drug-mucosal contact thereby encouraging absorption. In contrast, the more dehydrated viscous stool delivered to the left side of the colon may effectively sequester the drug, reducing mixing, drug- mucosal contact, and hence absorption. In the experiments to be described we have manipulated the intracolonic water content by predosing volunteers with either lactulose, a non-absorbable osmotic laxative, or the antidiarrhoeal drug codeine, to increase and decrease the percent of stool water respectively. This has allowed us to observe the effect of water content on the absorption of various probe molecules.

METHODS

Subjects

Sixteen healthy volunteers (8 male; 8 females), age range 20–40, were recruited into a 3 way randomised cross-over study comparing control values of absorption of a transcellular and paracellular probe with those observed while taking lactulose and codeine. 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, foods with known laxative properties (curries etc), and aspirin or non-steroidal anti-inflammatory 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 Ethics Committee and Administration of Radioactive Substances Advisary Committee (ARSAC) of the Department of Health.

Study Protocol

The study protocol is outlined in the diagram (Fig. 1). On the four days before the study day, and on the study day itself, volunteers were dosed with either lactulose 20 mls tds (Lactulose solution BP, 3.35 g/5 ml, Lagap, Hampshire, UK), codeine 30 mg qds, or no treatment, and were required to adhere to a 20 g fibre diet during these periods. On the evening before the study day (4th day of treatment) or the morning of the study day (5th day), the volunteers were required to produce a stool sample. This was subsequently stored at −20°C prior to freeze-drying to determine stool water content. Subjects were then dosed at 0800 h with a delivery system (Pulsincap™, Scherer DDS) comprising a slowly hydrating hydrogel plug, and a

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Table I. Table Showing Median Percent Values (Range) for the Urinary Excretion of Quinine According to Treatment and Site of Release

	Small intestine	Ascending colon	Transverse colon
Lactulose	7.2 (2.6–9.5) n = 6	3.2 (1.1–8.2)† n = 7	n/a
Control	4.4 (2.6–11.2)* n = 5	2.3 (1.3–3.0)*† n = 5	1.5 (0.6–3.8)* n = 6
Codeine	2.3 (0.9–24.4) n = 5	1.2 (0.4–3.7)† n = 8	n/a

Note: Excretion tended to be greater within any particular region for lactulose > control > codeine, and greater proximally for each particular treatment. Row (*) and column (†) showed significant trends (Jonckheere test for ordered alternatives).

water insoluble capsule whose contents were released at times dependent on the dimensions of the plug. The capsular contents were two marker probes, quinine and ^{51}Cr-EDTA (ethylenediamine-tetraacetic acid), and the non-absorbable imaging agent ^{111}In-labelled amberlite resin. Quinine dihydrochloride, a drug which rapidly crosses epithelial cells, was chosen as a marker of transcellular permeation, and ^{51}Cr-EDTA, a large water soluble, lipophobic molecule, was chosen to act as a marker of paracellular permeation.

In order to prevent premature activation of the hydrogel plug of the Pulsincap™, the ^{51}Cr-EDTA had to be introduced into the vehicle as a dry powder. This was achieved by drying a known activity of ^{51}Cr-EDTA solution onto a known weight of sucrose, and then adding the appropriate quantity of 'labelled' sucrose (equivalent to 1.8 MBq) into the delivery system. This method gives a consistent mean activity in the unit with a standard deviation of counts administered of 6% (n = 6). Images were obtained on the gamma camera at 30 minute intervals until 6 hours after the expected release time. Subjects were then allowed home before returning for a final scan 20 hours after the expected release time. Blood samples were obtained throughout the study day for later analysis of quinine content, and urine was collected for 20 hours from expected release time to give serial information about drug absorption from the lower gastrointestinal tract.

Pulsincap™ Delivery Systems

In order to target probes to the ascending colon, the Pulsincap™ delivery systems were manufactured to release their constituents at either 5, 6, or 8 hours after dosing dependent upon the treatment arm of the study. Our previous experience had suggested that a 6 hour release time would provide the most reliable delivery to the ascending colon whilst on no treatment. A faster 5 hour release Pulsincap™ was chosen in anticipation of the more rapid intestinal transit induced by lactulose, and an 8 hour release Pulsincap™ for the slower intestinal transit in the codeine arm of the study. Thus by targeting a particular region of the colon, and manipulating its milieu, the effects of differing stool water content on colonic absorption could be assessed.

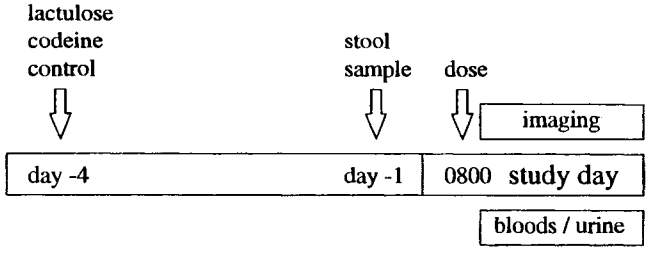

Fig. 1. Study protocol.

Scintigraphic Imaging

Scintigraphic images were obtained using an IGE Maxicamera fitted with a medium energy collimator (300 keV max) and set with a 20% window for dual acquisition of the 140-keV radiation peak of ^{99m}Tc and the 247-keV radiation peak of ^{111}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 ^{99m}Tc) anteriorly and posteriorly over the hepatic area.

Scintigraphic Analysis

The position and time of release of markers from the delivery system was determined by visual inspection of the serial images. Release was readily apparent from the serial images as a rapid increase in the area and concomitant decrease in the intensity of the hot spot. The position of the intact Pulsincap™, or geometric centre (GC) of the released isotope, were determined using a modification of the method described by Krevsky (5). In brief the colon was divided into 8 areas and the GC calculated from the formula $GC = \Sigma_1^8 Cn \times n/\overset{8}{\underset{1}{\Sigma}} Cn$. This gives a number between 0 and 9 which approximates to the midpoint of the isotope distribution. The values in this study ranged from 0–9: 0, small intestine; 1, terminal ileum; 2&3, lower and upper ascending colon; 4&5, right and left transverse colon; 6+7, upper and lower descending colon; 8, rectosigmoid colon, and 9 excreted. An index of spread (or dispersion) was created by calculating the average number of regions containing 10% or more of the original activity, following scintigraphically determined release from the delivery system. Thus, once release was seen to occur, the number of regions involved in each subsequent view was summed and divided by the total number of views taken following release. A dispersion score was therefore determined in each subject for all 3 arms of the study.

Stool Water Content

Stool samples from all subjects in each of the three arms of the study, produced on either day 4 or 5 of the treatment period, were collected into air tight plastic containers and stored at −2°C in sealed plastic bags. At the end of the study, aliquots (of approximately 5–10 grams) of the stool were freeze dried for 24 hours in order to determine their percent water content.

Quantification of Marker Probes

The analysis of quinine was carried out using an established reversed-phase HPLC method (6). 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 3mM tetrabutylammonium bromide (TBA) and 20 mM sodium dodecyl sulphate (SDS), pH 2.5. The stationary phase consisted of a Hypersil C-18 column (5mm) 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 ^{51}Cr-EDTA content. Reference standard solutions of ^{51}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.

Statistical Analysis

Treatment effects were assessed using non-parametric tests: Wilcoxon Paired Rank Tests or the Sign Test for paired comparisons, and trends were analysed using the Jonckheere test for ordered alternatives, thus avoiding the need for assumptions concerning the normality of distribution of the data.

RESULTS

All volunteers completed the 3 arms of the study, apart from one individual who missed the codeine treatment arm due to an intercurrent illness. No adverse effects were reported.

Pulsincap™ Delivery Systems

Scintigraphically the delivery systems were judged to have successfully released their contents in all cases apart from in two individuals in the codeine arm of the study. Subsequent HPLC measurements of quinine in blood samples showed that in one of these two cases release had in fact occurred but not been appreciated because of negligible dispersion following release.

The 5 hour release Pulsincaps™ were seen to release their contents at 5.2 ± 0.2 hours (mean ± SEM)(range 4.0–6.0). Comparative values for the 6 hour release Pulsincap™ were 6.9 ± 0.6 hours (range 5.5–9.5), and for the 8 hour release Pulsincap™, 7.7 ± 0.8 hours (range 6.0–10.5).

Sites of Release

Interindividual variability in whole gut transit times resulted in a spread of initial release sites in each of the treatment groups. The 3 different predicted release times of the Pulsincap™ delivery systems however, compensated for the overall differences in transit induced by lactulose, codeine or control diet, and resulted in a similar spread of initial release within the 3 groups. Thus, in the lactulose arm of the study, initial release occurred in the small intestine (6), ascending colon (7), and transverse colon (1), with release in one case in the stomach and the descending colon. In the control arm, release occurred in the small intestine (5), ascending colon (5), and transverse colon (6), and in the codeine arm in the small intestine (5), and the ascending colon (8), with release in the descending colon in one case and no release in the other individual.

Transit

As expected lactulose accelerated transit, as evidenced by a greater geometric centre value (4.8 ± 0.4) at 11 hours post dose (the final image point in the lactulose arm on the study day) compared to codeine (2.8 ± 0.3), $p < 0.001$, and the control treatments (3.9 ± 0.3), $p = 0.03$. Ascending colon transit time, calculated as the time for 50% of activity to pass beyond the hepatic flexure minus the time for 50% to enter the colon, was also significantly faster on the lactulose treatment (3.7 ± 0.8 hours) compared to codeine treatment (8.9 ± 1.8 hours), $p < 0.01$. In the lactulose treatment arm 10/15 (66%) subjects had defaecated a proportion of the delivered isotope by the end of the urine collection (20 hrs post expected release), compared to 8/16 (50%) in the control arm, and just 3/14 (21%) in the codeine arm. Hence there was relatively less probe marker available for permeation in the lactulose arm compared to the control and codeine arms of the study, a factor to be borne in mind when comparing overall absorption.

Stool Water Content

Lactulose significantly increased stool water content (75 ± 2%) compared to both the control values (71 ± 2%, $p < 0.01$), and codeine treatment (61 ± 2%, $p < 0.01$), as assessed by freeze drying stool samples (Fig. 2).

Dispersion of ^{111}Indium-Labelled Amberlite Resin

Dispersion, as defined by the average number of regions containing at least 10% of the total activity following release,

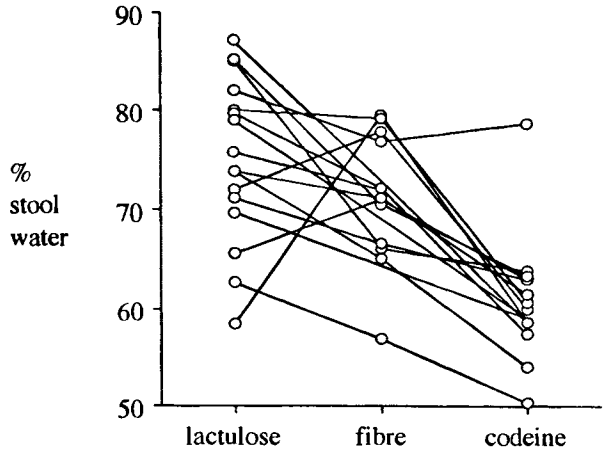

Fig. 2. Graph showing water content of stool following 4 days ingestion of lactulose, 20 gm fibre diet (control), or codeine.

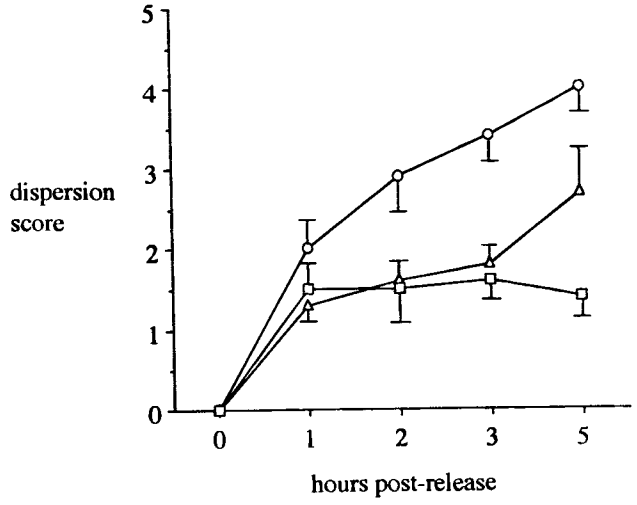

Fig. 3. Dispersion of material following release from timed release delivery system. Dispersion score indicates number of regions of colon containing ≥10% of total activity present in colon (circles = lactulose; triangles = control; squares = codeine).

was significantly greater at 3 hours following lactulose treatment (3.4 ± 0.3, n = 15) compared to both codeine (1.6 ± 0.2, n = 11), p = 0.04 and control values (1.8 ± 0.2, n = 14), p < 0.01 (Fig. 3).

Permeation of Marker Probes

Overall, regardless of site of release, absorption of quinine was significantly greater in the lactulose arm (n = 16) compared to the control arm (n = 16), as assessed by the 0–20 hr urine collection (4.66 ± 0.78% vs 3.02 ± 0.63%, p < 0.05). By contrast, there was a trend for reduced quinine absorption in the codeine (n = 13) compared to the control arm (2.60 ± 0.77 vs 3.02 ± 0.63%, p = 0.20) (Fig. 4).

There was a significant trend when looking at time to reach peak plasma concentration, Tmax, (time to peak value minus time immediately prior to first appearance in plasma) with the different treatments (and hence stool fluid content). Tmax was the most rapid in the lactulose arm (median = 1.50 hrs; range 0.50–3.25) followed by the control arm (median = 2.88 hrs; range 0.75–17.25), and slowest in the codeine arm (median = 4.25; range 1.50–16.50), p = 0.001, n = 12, (Pages Test for trend). A similar trend did not exist when peak plasma concentrations were examined, although the lactulose arm showed the greatest values (median; [range]): lactulose = 0.86 μg.ml; [0.30–4.77]; control = 0.23 μg.ml; [0.08–0.86]; codeine = 0.26 μg.ml; [0.16–1.48].

With regard to release in the ascending colon specifically, absorption of quinine (as assessed by 0–20 hr urine collection) was significantly enhanced by increasing luminal water content. Thus median percent absorption following codeine pretreatment was 1.2% (n = 8), control values were 2.3% (n = 5) and after lactulose pretreatment 3.2% (n = 7), (p < 0.01, Jonckheere test for ordered alternatives, Table I). In 5 individuals release occurred in the ascending colon in both the lactulose and codeine arms of the study, and in each case quinine absorption was greater in the lactulose arm (3.66 ± 1.43% vs 1.01 ± 0.25%, p = 0.03, n = 5, Fig. 5). Quinine plasma profiles following release in the ascending colon in these 5 paired samples suggested a more rapid upstroke and greater peak concentrations following lactulose pretreatment, but the data set (n = 5) was too small to show significant differences (Fig. 6).

A similar pattern of increasing quinine absorption with increasing luminal water content was also seen when release occurred in the small intestine (codeine 2.3% [n = 5], control 4.4% n = [5], lactulose 7.2% [n = 6], table). This trend however was not significant due to one outlier in the codeine group (24.4%).

In contrast to the data observed with quinine, no permeation gradient was seen for the paracellular probe, EDTA, when comparing the lactulose, control and codeine arms (0.76% vs 0.64% vs 0.80% medians, respectively) (Fig. 7).

DISCUSSION

One of the chief functions of the colon is resorption of sodium and water. It is capable of resorbing up to 6 litres of fluid per day (7), which results in the stool becoming progressively more dehydrated during its passage through the colon.

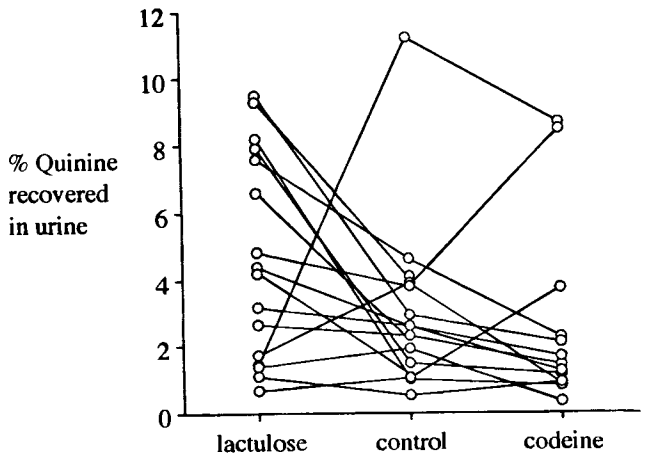

Fig. 4. Graph showing percent of ingested quinine appearing in a 0–20 hr urine collection following the different pretreatments (single extreme outlier, 24.4%, in the codeine arm excluded).

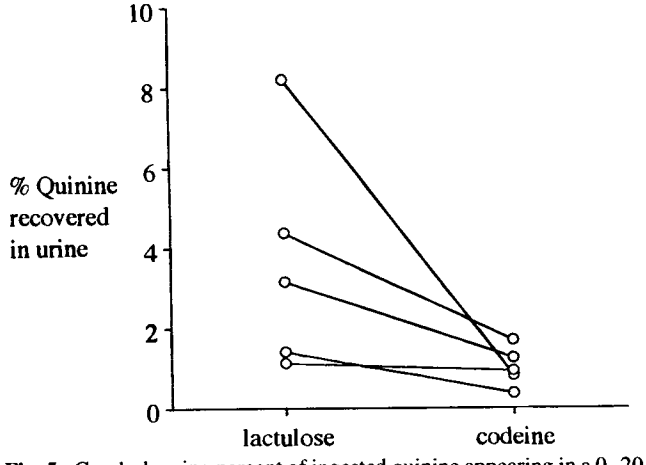

Fig. 5. Graph showing percent of ingested quinine appearing in a 0–20 hr urine collection for the 5 individuals who released in the ascending colon in both the lactulose and codeine arms of the study.

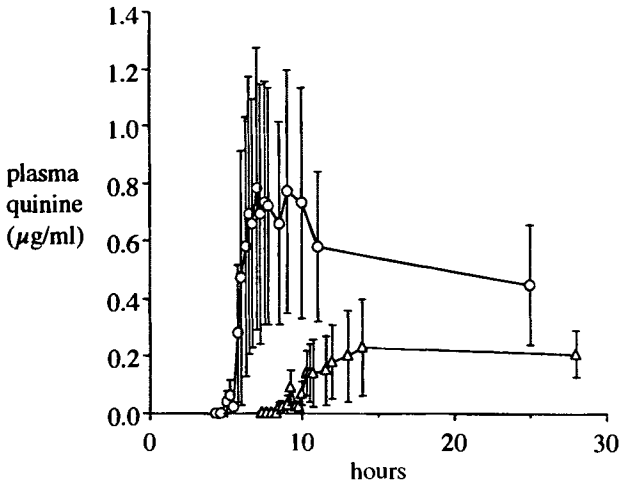

Fig. 6. Plasma profile of quinine concentration in the 5 pairs of individuals who released in the ascending colon in both the lactulose (circles) and codeine (triangle) treatment arms. Note that upstroke for lactulose starts at 5 hours (i.e., release time) and is steeper and reaches a higher peak concentration compared to codeine (starting at 8 hours, i.e., release time). Vertical bars indicate standard error of the mean.

There is therefore a marked difference in the milieu of the right and left colon, with the former containing liquid chyme and the latter viscous dehydrated stool. Data on regional absorption from the colon is sparse. A gradient favouring proximal colonic absorption has been demonstrated for the antibiotic ciprofloxacin (1) and preliminary results from our own centre suggested an aboral gradient for permeation of transcellular and paracellular marker probes, quinine and EDTA respectively (2). Electrophysiological studies showing that tight junctions become progressively less permeable in the aboral direction in the colon (3) may contribute to this observed gradient, as may the barrier afforded by the mucus layer which becomes thicker distally (8). We proposed however that the main factor influencing colonic absorption is the water content of the luminal material.

Our data shows that lactulose treatment significantly enhances absorption of the transcellular marker probe, quinine, following delivery to the distal small bowel and ascending colon. This occurs despite the faster transit which reduced residence time within the colon. We have also demonstrated that lactulose treatment increases the water content of the stool and results in greater dispersion of the released marker probe. The greater spread promotes mucosal contact and would be expected to optimise absorption.

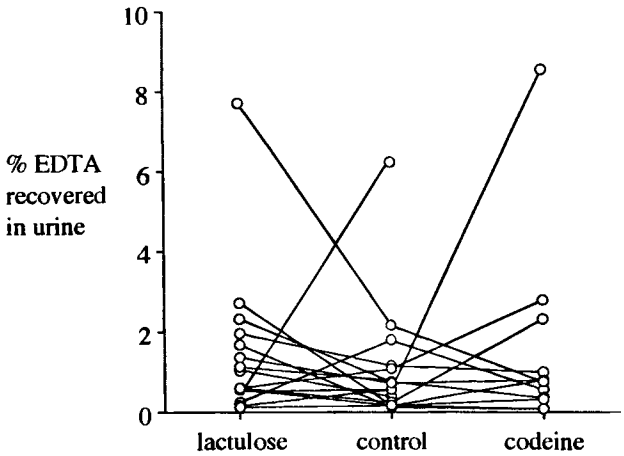

Fig. 7. Graph showing percent of ingested ^{51}Cr-EDTA appearing in a 0–20 hr urine collection following the different pretreatments.

Pretreatment with codeine resulted in reduced absorption of quinine from the colon compared with lactulose. This reduction occurred despite slower transit which reduced the proportion of released material excreted from the body hence effectively increasing the quantity available for absorption. Codeine also caused the released material to be retained in the more proximal regions of the colon whose epithelium may be more permeable compared to the distal colon (3). However, in direct contrast to the lactulose treatment arm, stool water and dispersion of released material were both reduced, discouraging drug mucosal contact and providing a possible mechanism to explain the opposing effects on quinine absorption.

An alternative explanation for the differential effects of lactulose and codeine on the absorption of quinine, resides in their potential effects on motility and hence mixing and dispersion of formulations, and consequent mucosal contact. In this context, codeine has pronounced antiperistaltic activity, and lactulose although predominantly thought to work as an osmotic laxative may also stimulate peristalsis following bacterial fermentation to short-chain fatty acids (10).

The results therefore show that manipulation of the luminal water content of the distal gut may have an important influence on the absorption of the transcellular marker probe quinine. We achieved these differences by pharmacological methods using lactulose and codeine. Progressive water resorption by the colon results in a parallel situation in the normal right and left colon, and may be the critical factor determining the proximal-distal gradient of absorption. Indeed, in the control arm of our study, progressively more distal delivery of quinine resulted in less absorption.

Surprisingly no such gradients were seen for the paracellular probe, EDTA. A possible reason for this may lay in the fact that several EDTA absorption values seen in this study far exceed those reported following oral ingestion of an ^{51}Cr-EDTA solution (10–12). One explanation of these results is that pulsed delivery of sucrose and ^{51}Cr-EDTA may result in high local concentrations which alter local permeability at the site of release, and hence confound any true differences in absorption by treatment. Alternatively, the relative paucity of the paracellular pathways (compared to the transcellular routes) may be rate determining and therefore minimise the influence of hydration of the luminal material.

In summary, the present study shows that lactulose enhances the permeation of the transcellular probe quinine in the distal gut. This effect may be mediated by increased luminal water content or by enhanced motility in this region, both of which would promote dispersion of formulation and therefore luminal contact. The more fluid environment of the right colon is therefore likely to be better suited to the permeation and absorption of formulations of low molecular weight, soluble drugs like quinine, compared to the distal colon.

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