Comparison of oro-caecal transit times assessed by the lactulose/breath hydrogen and the sulphasalazine/sulphapyridine methods

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SUMMARY The lactulose/breath hydrogen and the sulphasalazine/sulphapyridine methods of assessing oro-caecal transit time have been compared. In a two part crossover study in healthy normal subjects the median oro-caecal transit time by the SLZ/SP method was 4.84 hours but only 2.92 hours by the lactulose/breath hydrogen method. Co-administration of lactulose and sulphasalazine to nine subjects with assessment of oro-caecal transit time by hydrogen breath determination and plasma sulphapyridine assay gave oro-caecal transit times of 2.33 and 2.25 hours respectively suggesting that the lactulose reduces transit time and that the lactulose/breath hydrogen method, which is so convenient to use, gives artificially low transit times. A third experiment was undertaken to compare the oro-caecal transit times after 1.5 and 3.0 g sulphasalazine. The oro-caecal transit times after the two doses were not statistically different.

Traditional methods of investigating upper gastrointestinal transit include radiology, isotopically labelled test meals, and intubation. Intubation has been shown to inhibit gastric emptying and to speed up small bowel transit¹ and the use of radioopaque materials necessarily dictates meal composition. Isotopically labelled meals are of particular value but the associated radiation may present ethical problems. The presence of flora in the large bowel provides an alternative approach whereby use is made of bacterial metabolism. Thus conversion of lactulose (galactosido-fructose) by large bowel flora to short chain fatty acids together with small quantities of hydrogen gas provides such a method: the hydrogen gas by virtue of its low molecular weight and relative insolubility in water is rapidly absorbed into the blood, transported to the lungs, and exhaled in the breath.^{2,3} An alternative method originally described by Kennedy *et al*⁴ uses the azoreductase producing flora of the large bowel to split the azo link releasing 5-aminosalicylic acid and sulphapyridine from the orally administered sulphasalazine. The sulphapyridine is absorbed into the blood stream and

can be detected. By replacing the optical assay used by Kennedy with a more sensitive, high pressure liquid chromatographic (HPLC) assay we were able to obtain a useful measure of transit time. Kellow *et al*⁵ have recently shown that both the sulphasalazine/sulphapyridine method and the lactulose breath hydrogen method do measure oro-caecal transit time. I have been using both methods since 1980 to study oro-caecal transit. My experience with the two tests is summarised in Table 1. It was observed that the transit time obtained by use of the sulphasalazine/sulphapyridine method tended to give longer transit times than those found by the lactulose/breath hydrogen technique. The three experiments reported here were devised to compare the two methods, in the same subjects, and to ascertain whether the sulphasalazine itself affects transit time.

Methods

SUBJECTS

All studies were subject to ethics committee review and informed consent was given. The participants were healthy normal subjects not patients and were not currently taking any medication. They attended after an overnight fast and were in a semirecumbent

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Table 1 *Transit time in hours obtained in the placebo/control phase of a series of 36 two part crossover studies. Some studies called for administration of the sulphasalazine or lactulose with a standard rice pudding test meal. The oro-caecal transit time was measured in 275 healthy subjects by the lactulose/breath hydrogen method and in 64 subjects by the sulphasalazine/sulphapyridine method. The transit time found using the sulphasalazine/sulphapyridine method is longer than that measured by the lactulose/breath hydrogen method whether or not the lactulose or sulphasalazine was given with a test meal*

	Aqueous preparation	Rice pudding
Lactulose/breath hydrogen method	1.60 (0.57)	2.27 (0.80)
Sulphasalazine/sulphapyridine method	3.40 (0.93)	4.63 (1.25)

position throughout the experiment. Three investigations are here reported:

A CROSS OVER STUDY TO COMPARE TRANSIT TIME ASSESSED BY THE LACTULOSE/BREATH METHOD AND THE SULPHASALAZINE/SULPHAPYRIDINE METHODS

Eight healthy male subjects of mean age 25.9 (9.1) yr and weight 78.2 (11.9) kg took part in a double blind two part crossover study. They attended in the fasting state and were semirecumbent for the first four hours of the study. They had abstained from beer and those foods rich in non-utilisable sugars – for example, beans, during the previous 24 hours. Before giving the test meal breath samples were taken for determination of hydrogen content every 10 minutes for one hour, the mean of these readings providing the baseline value. Breath samples were then taken at 10 minute intervals until a rise of 15 ppm above the baseline value was noted; this being the end point. If a rise was noted of less than 15 ppm the frequency of breath sampling was increased to every five minutes. Breath samples were taken at end expiration using a T-tube connected to 1.5 m Silastic tubing (6 mm id). Samples were taken with a 20 ml syringe and assayed for the hydrogen content immediately. Lactulose (20 ml lactulose solution BP) or sulphasalazine (2 g) was admixed with 200 g rice pudding (Commercial tinned rice pudding (Ambrosia), was used for consistency between experiments). Colouring was added to make the test meals similar in appearance. Breath samples were taken for 180 minutes after the test meal and blood samples were collected from one hour 40 minutes at 20 minute intervals until five hours then hourly until eight hours post-test meal from each person in both parts of the study.

ASSESSMENT OF OROCAECAL TRANSIT TIME BY BOTH THE LACTULOSE/BREATH HYDROGEN AND THE SULPHASALAZINE/SULPHAPYRIDINE METHODS WHEN THE LACTULOSE AND SULPHASALAZINE WERE COADMINISTERED

Nine healthy male subjects of mean age 24.0 (7.7) yr and weight 70.6 (12.9) kg took part in this study, each receiving a rice pudding test meal (200 g) containing both lactulose (20 ml lactulose solution BP) and sulphasalazine (2 g). They attended in the fasting state and were semirecumbent for four hours. Expired breath and blood samples were collected at the same intervals as in the previous study.

TO ASCERTAIN WHETHER SULPHASALAZINE ITSELF HAS ANY EFFECT ON OROCAECAL TRANSIT TIME

Nine healthy subjects (four women) of mean age 24.9 yr and weight 70.9 kg took part in this two part crossover study. Each person received 1.5 g sulphasalazine on one visit and 3.0 g on the other. The sulphasalazine was given with a rice pudding test meal as above and the subjects were semirecumbent. Blood samples were taken for SP assay from one hour 40 minutes at 20 minute intervals until five hours then hourly until eight hours post dose.

ASSAY OF SULPHAPYRIDINE

An HPLC method was used.⁶ This uses a 25 cm C-18 Bondapak column with 1% acetic acid and methanol (77:23) as eluent at a flow rate of 2.5 ml/min. Ultraviolet detection at 270 nm was used. The first appearance of sulphapyridine in plasma, which rises along a sigmoid curve, was taken as indicating the oro-caecal transit time.

MEASUREMENT OF BREATH HYDROGEN

Hydrogen gas concentration in end expired breath samples was determined using an electrochemical cell (Gas Measurements Ltd, Renfrew, Scotland) which was calibrated each day using a hydrogen meter has an accuracy of $\pm 2\%$ and a repeatability of ± 1 ppm.

Results

A CROSSOVER STUDY TO COMPARE TRANSIT TIME ASSESSED BY THE LACTULOSE/BREATH METHOD AND THE SULPHASALAZINE/SULPHAPYRIDINE METHODS

The plasma concentrations of sulphapyridine were measured at the times specified above and the time of first appearance of the sulphapyridine taken as indicating arrival of the head of the test meal at the caecum. The oro-caecal transit times so obtained together with the oro-caecal transit times when

Table 2 Table of transit times: Results of a double blind crossover study to compare the determination of oro-caecal transit time by the lactulose/breath hydrogen method on one occasion and by the sulphasalazine/sulphapyridine method on another

Subject no	Orocolonic transit time	
	Lactulose/breath hydrogen time (h)	SLZ/SP method time (h)
1	2:83	4:67
2	3:00	5:00
3	2:00	4:67
4	3:67	4:33
5	2:17	7:00
6	3:17	5:00
7	3:00	3:67
8	2:75	6:00
Median (range)	2:92 (2:0-3:67)	4:84 (3:67-7:0)

These results are significantly different by application of the Wilcoxon's signed-rank test ($p=0.01$); SLZ/SP=sulphasalazine/sulphapyridine.

assessed by the lactulose/breath hydrogen method are given in Table 2 above. The median oro-caecal transit time by the lactulose/breath hydrogen method was 2:92 hours but by the sulphasalazine/sulphapyridine method 4:84 hours; all eight subjects having longer transit times when this was measured by the sulphasalazine/sulphapyridine method. The difference was significant by the Wilcoxon's signed-rank test with $p=0.01$.

ASSESSMENT OF OROCAECAL TRANSIT TIME BY BOTH THE LACTULOSE/BREATH HYDROGEN AND THE SULPHASALAZINE/SULPHAPYRIDINE METHODS WHEN THE LACTULOSE AND SULPHASALAZINE WERE COADMINISTERED

The result of measuring simultaneous oro-caecal transit times when the lactulose and sulphasalazine were coadministered are given in Table 3. In this exercise each of the nine subjects had only one test meal but this contained both lactulose and sulphasalazine. The median transit times assessed by appearance of hydrogen and sulphapyridine were 2:25 and 2:33 hours respectively, results which were not significantly different. Seven of the nine subjects showed reasonable correlation in the two times with a mean difference of 15 minutes. Subject 17 had a rather slow rise in hydrogen levels which might reasonably have been judged to indicate a shorter transit time but for the purpose of this exercise the strict criterion of a rise of 15 ppm was adhered to. Subject 3 had some sulphapyridine present at one hour 40 minutes but not in the two hour sample, samples thereafter rising rapidly so two hours 20 minutes was taken as the oro-caecal transit time.

Table 3 Determination of oro-caecal transit time in a group of healthy subjects who received a test meal containing both lactulose and sulphasalazine. The oro-caecal transit times were measured both by assay of breath hydrogen and plasma sulphapyridine. The oro-caecal transit times so determined were not significantly different by application of the Wilcoxon's signed-rank test

Subject no	OroCaecal transit time	
	Lactulose/breath hydrogen time (h)	SLZ/SP method time (h)
9	3:00	3:00
10	3:00	2:67
11	1:00	2:33
12	1:92	1:67
13	1:92	1:67
14	3:33	2:67
15	2:25	2:33
16	2:67	2:33
17	3:50	2:33
Median (range)	2:25 (1:0-3:50)	2:33 (1:67-3:0)

TO ASCERTAIN WHETHER SULPHASALAZINE ITSELF HAS ANY EFFECT ON OROCAECAL TRANSIT TIME

The oro-caecal transit time after administration of 1.5 or 3 g sulphasalazine to the nine subjects is given in Table 4. The median oro-caecal transit time after the 1.5 g and 3 g doses were 4:67 hours and 4:00 hours respectively with six of the nine subjects having a longer oro-caecal transit time with the smaller dose. Application of a Wilcoxon's test to these data, however, indicate that this difference was not statistically significant and so provides no grounds for rejecting the null hypothesis that sulphasalazine itself does not affect the oro-caecal transit time over the range studied.

Table 4 Oro-caecal transit time times after 1.5 or 3.0 g sulphasalazine are shown for nine healthy subjects who took each dose in a two part crossover study. The median transit times are not significantly different by application of the Wilcoxon's signed-rank test

Subject no	Orocolonic transit time by the SLZ/SP method (h)	
	Dose of SLZ 1.5 g	Dose of SLZ 3.0 g
18	4:67	4:33
19	4:67	4:67
20	4:00	3:33
21	4:67	4:00
22	4:33	4:00
23	5:00	5:00
24	3:33	4:00
25	4:67	4:00
26	5:00	4:67
Median (range)	4:67 (3:0-5:0)	4:00 (3:33-5:0)

Discussion

The double blind comparison of the lactulose/breath hydrogen with the sulphasalazine/sulphapyridine methods of measuring oro-caecal transit time confirms the impression, gained from previous experience with the techniques, that the sulphasalazine/sulphapyridine method indicates a much longer oro-caecal transit time. In the crossover study reported here the transit time by the lactulose/breath hydrogen method was 60% of the obtained by the sulphasalazine/sulphapyridine method.

When the lactulose was coadministered with sulphasalazine it was found that the oro-caecal transit time assessed by the two methods was the same, and furthermore the transit time so measured is that expected with lactulose alone. It seems unlikely that the difference found between the two methods can be explained by a difference in the anatomical end point for the head of the test meal. The fact that the lactulose/breath hydrogen method measures an oro-caecal transit time is suggested by the original work of Bond and Levitt and by the simultaneous appearance of breath hydrogen and caecal radioactivity.⁷ The fact that both the lactulose/breath hydrogen and the sulphasalazine/sulphapyridine methods measure the same transit time is suggested by the simultaneous appearance of the respective metabolites after caecal instillation.⁵

These results suggest that administration of lactulose, even in small doses, alters the transit time one is attempting to evaluate. There is known to be an inverse relationship between transit time measured by the lactulose/breath hydrogen method and the amount of lactulose given,² however, the values we obtained with the sulphasalazine/sulphapyridine technique are greater than those reported for even 5 g lactulose. As lactulose passes the small intestine totally unabsorbed⁸ the mechanism by which it alters oro-caecal transit time is likely to involve retention of water by osmosis or a change in permeability.

Existing methods of measuring transit time are far from perfect. Most methods involve the administration of special meals – for example, barium or isotopically labelled foods; each technique dictating its own particular circumstances. The use of radio-opaque shapes has recently been described for studying colonic transit^{9,10} but is likely to be unhelpful for small bowel transit unless smaller markers can be used because non-digestible solids, greater than about 2 mm, are not emptied with the semi-solid component of a meal but must await the next interdigestive migrating motor complex.¹¹

The alternative flora dependent method, as described by Kennedy in 1979 used an assay¹² which was

insufficiently sensitive to be of practical value. Neither did we find adequate sensitivity using the improved optical assay described by Hansson and Sandberg,¹³ however, with an HPLC assay⁶ this becomes a useful technique. The sulphasalazine/sulphapyridine method was investigated at two doses, 1.5 and 3 g, and there was no statistically significant difference between the oro-caecal transit times measured. Kellow *et al.*,⁵ who recently described a different assay for the sulphapyridine, described the method as 'another simple measure of intestinal transit'. As a consequence of its practicality and simplicity the lactulose/breath hydrogen test soon became widely used, facts which may have caused a blind spot to its disadvantages. It is concluded that the lactulose/breath hydrogen method alters the transit time under study, whereas the sulphasalazine/sulphapyridine method appears free from this problem. It is suggested that the sulphasalazine/sulphapyridine method provides a truer measure of oro-caecal time. It is recognised that the sulphasalazine/sulphapyridine method is less convenient as it necessitates blood rather than breath sampling and the feasibility of measuring sulphapyridine in saliva is currently under investigation.

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