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Statistical analysis of the lactulose/breath hydrogen test in the measurement of orocaecal transit: its variability and predictive value in assessing drug action

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SUMMARY **The variability in the orocaecal transit time as measured by the lactulose/breath hydrogen method has been studied for three conditions: lactulose given with a meal, subjects sitting; lactulose given with a meal, subjects semirecumbent; lactulose given in aqueous solution, subjects semirecumbent. Thirty three healthy subjects attended on up to 12 occasions. It was found that administration of the lactulose with a meal significantly reduced the variability ($p<0.05$) and that adoption of the semirecumbent position further reduced variability. A power analysis was used to predict the number of subjects who would be required to show a given percentage change in orocaecal transit time at specified probabilities and powers. A graph and a table for use in the prediction of subject numbers at a probability of 5% and for powers of 50–99% is presented. A dose response curve for metoclopramide using the lactulose/breath hydrogen method is given for doses of 10, 15, and 20 mg.**

Small bowel transit time may be assessed radiologically, by radioisotope labelling of test meals and by use of intubation techniques, although these methodologies may themselves alter transit times and radiation exposure may present ethical problems. The presence of bacterial flora in the large bowel provides a range of possible alternative approaches. Thus the conversion of sulphasalazine to sulphapyridine by bacterial azoreductase with assay of this metabolite in blood,[1] the production of hydrogen from the raffinose and stachyose content of a baked bean meal or the hydrogen obtained from sorbitol[2] or lactulose (galactosido-fructose) have all been described.

The lactulose breath hydrogen (L/BH) test described by Bond and Levitt[3,4] is now in widespread use, but only limited application of the method to assess drug activity has been made.[5] The orocaecal transit time (OCTT), assessed by the L/BH method, is widely believed to be very variable and so before using this technique as a pharmacological tool, this variability must be quantified if statistically meaningful results are to be obtained.

When evaluating experimental data it is the usual statistical practice to assume that the treatment produced no change in the measurement of interest. This is the 'null hypothesis'. The null hypothesis is then challenged by an appropriate test, say a *t* test, and rejected or otherwise. Two sorts of error are possible. A type I error is made by rejecting the null hypothesis when it is in fact true – that is, concluding that the drug produced a change when in fact it did not. The probability of a type I error (the alpha level) is the probability with which the familiar p value is compared when a test is performed. A type II error is made by not rejecting the null hypothesis when it is in fact false. The probability of a type II error (the beta level) is closely related to the power (1-beta) of the test. Hence the power is the probability of detecting a difference when one really exists.

A compromise has to be made between the probabilities of the two types of error. Both types of error must therefore be considered in our evaluation.

In the investigations reported here the inter and intrasubject variation in small bowel transit time,

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assessed by use of the lactulose/breath hydrogen method, has been studied. Statistical analysis of these data have been undertaken to find the number of patients who would be required to show differences of specified magnitude at conventional levels of significance and power. This method has been found useful in assessing novel gastrokinetic agents and a dose response curve for metoclopramide is presented.

Methods

GENERAL METHODOLOGY

Normal healthy subjects attended on 12 occasions separated by intervals of not less than three days, usually weekly. They were fasting and had abstained from beer and those foods rich in non-utilisable sugars – for example, beans, during the previous 24 hours. After a 30 minute period to obviate the stress of travel, subjects were seated and breath samples taken for determination of hydrogen content every 10 min for one hour, the mean of these readings providing the baseline value. The lactulose (20 ml lactulose solution BP which has 3·35 g/5 ml) was then given either in water (50 ml) or rice pudding (220 g Ambrosia creamed rice) as required by the experiment. 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 (0·6 mm id). Samples were taken with a 20 ml syringe and assayed for the hydrogen content immediately.

ASSAY OF BREATH HYDROGEN

Breath hydrogen was assayed using an electrochemical technique. The hydrogen meter has an accuracy of ±2% and a repeatability of ±1 ppm (Gas Measurement Ltd, Renfrew, Scotland). The hydrogen meter was calibrated each day using a known source of 96 ppm.

INTER AND INTRASUBJECT VARIABILITY

Three separate experiments (1, 2, 3) were carried out to assess the inter and intrasubject variation in orocaecal transit time using the lactulose/breath hydrogen method with the conditions outlined in Table 1.

DOSE RESPONSE FOR THE ACTION OF METOCLOPRAMIDE ON SMALL BOWEL TRANSIT

Healthy normal subjects attended on four occasions and received metoclopramide (10, 15, or 20 mg as a syrup diluted to 50 ml or vehicle only as control) mixed with the lactulose containing rice pudding test meal. Other experimental conditions were as described above. Demographic data are given in Table 1 (experiment 4).

The studies reported here were approved by the West Middlesex University Hospital ethics committee. Subjects gave written informed consent.

Table 1 *Demographic information*

Experiment no		*Subject position*	*No of subjects (n)*	*Mean age and range*	*Mean weight and range (kg)*
1	Lactulose given with a meal	Sitting	11 6M, 5F	27 (18–39)	64 (36–93)
2	Lactulose given with a meal	Semi-recumbent	10 6M, 4F	29 (19–46)	64 (36–84)
3	Lactulose given in solution	Sitting	12 6M, 6F	28 (20–45)	63 (36–73)
4	Lactulose given with a meal	Semi-recumbent	16 14M, 2F	24 (19–39)	71 (50–93)

Results

INTER AND INTRASUBJECT VARIATION WITH THE LACTULOSE METHOD SITTING WITH A MEAL

The results are presented in Table 2 which shows the transit time in minutes for 11 subjects attending on 12 occasions.

INTER AND INTRASUBJECT VARIATION WITH THE LACTULOSE METHOD SEMIRECUMBENT WITH A MEAL

The results are presented in Table 3 which shows the transit time in minutes for 10 subjects attending on 12 occasions.

Table 2 *Results when lactulose was given with a meal of rice pudding and subjects were sitting*

Subject no	*Visit number* 1	2	3	4	5	6	7	8	9	10	11	12
1	65	90	100	110	100	100	80	90	90	80	105	95
2	75	105	100	115	100	120	150	95	190	120	115	100
3	95	85	100	100	125	120	170	115	110	190	140	115
4	120	155	120	130	135	110	90	135	100	130	135	140
5	90	120	90	65	90	120	85	120	80	115	140	115
6	120	110	90	80	90	60	80	80	85	90	80	95
7	70	75	110	85	80	55	70	115	140	120	90	70
8	110	60	80	85	80	90	90	100	80	95	100	75
9	90	115	50	140	90	120	150	130	110	160	160	105
10	80	80	70	95	130	90	80	115	190	100	100	120
11	170	140	140	120	100	90	90	95	110	90	110	160

Tables 2, 3, and 4 show the orocaecal transit times in minutes, assessed by the lactulose breath/hydrogen method, for groups of normal subjects who attended on up to 12 occasions.

Table 3 *Results when the subjects took the lactulose in rice pudding but were semirecumbent*

Subject no	Visit number 1	2	3	4	5	6	7	8	9	10	11	12
12	140	200	180	145	225	80	175	155	175	135	135	190
13	70	80	90	95	80	85	75	65	75	90	90	90
14	115	110	125	125	125	165	145	115	115	115	115	135
15	190	190	175	170	175	165	*	180	160	135	165	125
16	70	90	100	95	100	95	80	95	130	50	70	95
17	160	140	180	120	175	140	150	140	155	160	145	170
18	90	165	125	115	105	95	70	95	115	115	95	135
19	130	135	105	140	130	125	90	130	135	170	135	155
20	120	115	95	100	110	45	95	60	195	105	80	80
21	190	170	165	130	145	110	160	170	160	170	200	125

An * indicates when an OCTT could not be determined from the profile.

Table 4 *Results when lactulose was given in an aqueous solution and subjects were sitting*

Subject no	Visit number 1	2	3	4	5	6	7	8	9	10	11	12
22	50	60	105	90	50	110						
23	90	50	60	80	100	130	60	60	80	60	120	110
24	50	85	*	110	55	50	50	40	45	40	60	50
25	140	100	140	80	110	40	50	130	50	80	70	80
26	60	90	50	70	60	105	70	70	60	50		
27	70	120	50	90	110	110	90	50	70	85	60	65
28	65	65	65	80	90	110	70	110	110	70		
29	135	155	125	140	135	130	90					
30	*	70	50	70	40	125	80	60	70	110	60	40
31	60	40	50	50	60	40	50	30	60	70	55	110
32	80	80	80	50	90	105	110	100	80	65	80	90
33	150	40	50	60	100	60	90	90	45	60	90	80

An * indicates when an OCTT could not be determined from the profile.

INTER AND INTRASUBJECT VARIATION WITH THE LACTULOSE METHOD SITTING, AQUEOUS SOLUTION

The results are presented in Table 4 which shows the transit time in minutes for 12 subjects attending on 12 occasions.

ESTIMATION OF SAMPLE SIZE

It is desired to calculate the number of patients/subjects who would be required to show a given percentage change in orocaecal transit time at a level of significance and power which is statistically acceptable. The data on inter and intra subject variation presented in the tables above were first log transformed and then subjected to analysis of variance to obtain estimates of the relevant population variance for paired samples. The following formula enables calculation of the number of patients/subjects required using standard methods:[6 7]

$$n=(Z_{\alpha/2}+Z_{\beta})^2\cdot V/(\mathrm{Log}_{10}(1+^{P/100}))^2$$

Where Z is the normal distribution integral (found from tables or calculated by a formula.[8] Thus if the power selected was 80%, beta=0·2 and Z(beta)= 0·85. P is the percentage change.

The required population variance (V) obtained as described above is 0·01953 for patients sitting and lactulose given with a meal, 0·01623 for patients semirecumbent, lactulose given with a meal, 0·03712 for patients sitting and receiving lactulose in aqueous solution.

Finally a small correction is required[6] to compensate for the necessary use of the normal distribution to approximate to the ideal t distribution. Add 2 on to N for alpha values of 5%, and 3 for 1%.

Using this formula the number of subjects who would be needed to show percentage changes of 10 to 50% in transit time for probabilities of 5% and 1% at two statistical power levels have been calculated and are shown in Table 5 below. Figure 1 shows the number of subjects who would be needed to show percentage changes in the OCTT for a range of powers of (50–99%) at a probability of 5%.

Table 5 *Number of subjects needed to show a given percentage difference in orocaecal transit time at alpha levels of 1% and 5% for powers of 80% and 95%. Data for three experimental conditions are shown depending on patient position and whether the lactulose was administered with a meal or not*

		Lactulose given with a meal. Subjects sitting				*Lactulose given with a meal. Subjects semirecumbent*				*Lactulose given in solution. Subjects sitting*			
Power		*80%*		*95%*		*80%*		*95%*		*80%*		*95%*	
Alpha level		*1%*	*5%*	*1%*	*5%*	*1%*	*5%*	*1%*	*5%*	*1%*	*5%*	*1%*	*5%*
Per cent difference	10	137	92	207	151	114	77	172	126	257	173	390	284
	20	40	27	59	43	34	23	50	36	73	49	109	79
	30	21	14	30	22	18	12	26	19	37	25	54	40
	40	14	10	20	14	12	8	17	12	24	16	34	25
	50	11	7	15	11	10	7	13	9	17	12	25	18

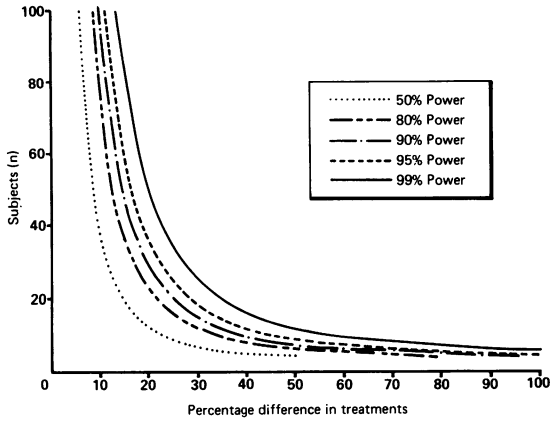

Fig. 1 *Number of subjects needed to show the given percentage changes in OCTT for a range of powers (50 to 99%) at an alpha level of 5%. Subjects being semirecumbent and receiving the lactulose with a meal.*

DOSE RESPONSE FOR THE ACTION OF METOCLOPRAMIDE ON SMALL BOWEL TRANSIT

The mean breath hydrogen concentrations after placebo or metoclopramide are shown in Figure 2 and the mean OCTT's are given in Table 6.

The orocaecal transit times found after the three doses of metoclopramide and placebo were subjected to a two way analysis of variance after a log transformation; $F(3,45)=10{\cdot}87$, $p<0{\cdot}001$. A similar analysis on times to peak hydrogen concentrations gave $F(3,45)=3{\cdot}064$, $p=0{\cdot}04$. Metoclopramide produced reductions in the orocaecal transit time of 21·6, 30·0, and 29·6% at doses of 10, 15, and 20 mg respectively.

Discussion

Many factors affect orocaecal transit, including all those which affect gastric emptying. Thus meal volume, amount and nature of any lipid content, presence of fibre, patient position, and thyroid or luteal state have all been implicated. Small bowel transit itself is known to show considerable variation from one person to another. Lonnerblad,[9] reviewing barium studies, commented on the great diversity observed. Seuk,[10] also using barium found[11] the mean small bowel transit to be less than two hours for 83% of cases but to range from 15 minutes to five hours.

The variability of OCTT when assessed by the L/BH method has been commented on by several workers. Bond and Levitt,[3] who developed the Lactulose/breath hydrogen method for the measurement of OCTT, studied six subjects on three to five occasions and described the reproducibility as 'relatively good'. La Brooy,[12] however, using a liquid lactulose test studied the intra and intersubject variation in up to 12 subjects on three occasions and found coefficients of variation of 18·5 and 29·7% with doses of 10 and 15 g respectively. They felt the test not to be reproducible in or between individuals in the form used. Ravich[11] who studied six subjects on three occasions felt that transit time when measured by the onset of the rise in hydrogen tension appeared

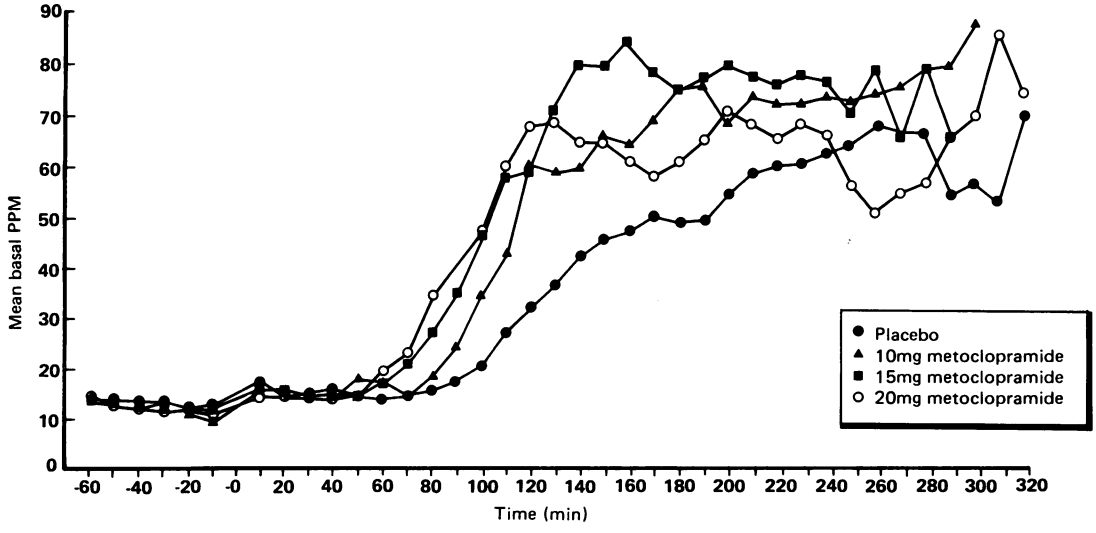

Fig. 2 *Mean breath hydrogen concentrations as a function of time for the 16 subjects who each received, in a crossover study, either placebo or metoclopramide 10, 15, or 20 mg before a lactulose containing meal.*

Table 6 *Analysis of metoclopramide dose response data*

	Orocaecal transit time mean (SD)	*Time to peak mean (SD)*
Control	129·1 (47·6)	216·6 (56·1)
Metoclopramide 10 mg	101·2 (26·5)	197·2 (48·0)
Metoclopramide 15 mg	90·3 (26·8)	184·4 (49·5)
Metoclopramide 20 mg	90·9 (41·7)	174·4 (56·3)

The mean and standard deviation of individual orocaecal transit times assessed by a rise in breath hydrogen concentrations. Metoclopramide 10, 15, 20 mg or placebo were administered with a lactulose containing test meal.

reasonable but found wide intersubject variation in the extent and duration of the rise.

When using the L/BH method to assess changes brought about in the OCTT by pharmacological agents or diet as many factors as possible need to be standardised and the cross over design used whenever practicable. The suggestion[12] that better reproducibility might be found by combining the lactulose with a meal is confirmed by the above studies. Comparison of the analyses of variance for the OCTT after administration of the lactulose with a meal as opposed to administration in aqueous solution shows the variability to be significantly reduced ($p<0·001$). Furthermore adopting the semirecumbent posture during the test, as opposed to sitting, provides further reduction in variability though this was not statistically significant ($p>0·05$).

In conclusion the variability in the lactulose/breath hydrogen test is not so great as to prevent the use of the method in assessing the action of drugs in suitably designed crossover studies using relatively small groups of patients if these are likely to produce an effect of size sufficient to have clinical significance. The dose response study for metoclopramide illustrates this; with 16 subjects activity at, at least, the 5% level of significance was shown. The practical implication of this work is that having quantified the variability in the OCTT when measured by the L/BH method the number of patients/subjects who should be included in a study to produce statistically useful results can now be forecast provided one has an idea of the magnitude of the change expected.

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