Simplified oral pancreatic function test

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SUMMARY The standard Bentiromide test and a new modified test using p-aminosalicylic acid (PAS) as a pharmacokinetic marker for p-aminobenzoic acid (PABA) have been evaluated in the detection of pancreatic exocrine insufficiency in children. The conventional two day test using a colorimetric assay for urinary PABA discriminated poorly between five children with pancreatic insufficiency and 13 others with normal pancreatic function. Two further groups of patients, comprising 28 with pancreatic exocrine insufficiency and 20 with normal pancreatic function underwent the modified test, and urine samples were analysed by high performance liquid chromatography. The results showed a complete separation between groups. The use of PAS eliminates a number of sources of error inherent in a two day Bentiromide test and provides a simplified and accurate diagnostic test for pancreatic insufficiency. The PABA-PAS modified test enables collection of the urine to be done during a single six hour period.

Exocrine pancreatic insufficiency is often considered in the differential diagnosis of failure to thrive, protracted diarrhoea, or steatorrhoea. Though it is most commonly seen in patients with cystic fibrosis, it may occur in a number of other disorders.¹ Duodenal intubation followed by hormonal stimulation of the pancreas with intravenous pancreozymin and secretin is still considered the most reliable test for the diagnosis of pancreatic exocrine insufficiency. This test is, however, unpleasant for the patient, subsequent laboratory analysis is both time consuming and complex, and reliable supplies of secretin are difficult to obtain.

Simple screening tests for pancreatic insufficiency have therefore been developed and evaluated largely in adult patients as alternatives to duodenal intubation.² The Bentiromide test has been most widely evaluated and relies on hydrolysis of an orally administered synthetic compound N-benzoyl-L-tyrosyl p-aminobenzoic acid (Bentiromide) within the small bowel. This compound is cleaved selectively by pancreatic chymotrypsin to release p-aminobenzoic acid (PABA). PABA is absorbed in the small intestine, conjugated in the liver, and excreted in the urine. The amount recovered from a timed urine collection expressed as a proportion of the PABA given in the dose of Bentiromide gives an indication of duodenal chymotrypsin activity and hence of exocrine pancreatic function. False positive results may occur, however, in patients with dis-

ordered gut motility or an enteropathy (which result in low PABA absorption) or in those with hepatic or renal dysfunction, when either conjugation or urinary excretion of PABA may be reduced.³ In addition, drugs such as paracetamol and sulphonamides, or certain foods, may interfere with the urinary assay of PABA.

An attempt to correct for these variables has been reported whereby a dose of free PABA alone is given the day after the Bentiromide test. A PABA excretion index is then derived by dividing the amount of PABA recovered on day 1 by the amount recovered on day 2. This makes the test more cumbersome but can improve specificity⁴ by reducing the number of false positives. Subsequent refinements have included administration of PABA labelled with ¹⁴C at the same time as the Bentiromide,⁵ which avoids the need to repeat the test on a second day. Though it is undesirable to give the β emitter ¹⁴C to children in a diagnostic test, the single day test with ¹⁴C PABA has gained acceptance as a screening test for pancreatic disease in adults.⁶

As an alternative to ¹⁴C PABA we have evaluated the use of a structural analogue of PABA, p-aminosalicylic acid (PAS), and compared the initial PABA excretion index obtained with ¹⁴C PABA with that using PAS. The advantage of using PAS is that it is structurally and pharmacokinetically related to PABA and is safe. Furthermore, after low

doses, its concentration in urine can be measured simultaneously with PABA by high performance liquid chromatography. The comparison of PABA excretion index obtained using ^{14}C PABA and then PAS in a group of adult patients with a wide range of pancreatic exocrine function showed a high correlation ($r=0.96$) confirming the pharmacokinetic similarities of PABA and PAS.⁷

We have therefore assessed the ability of the test incorporating PAS to discriminate between normal and abnormal pancreatic function in children, and have compared our experience with the conventional two stage Bentiromide and free PABA test.

Subjects and methods

UNMODIFIED TWO STAGE TEST

Five children with clinically apparent pancreatic insufficiency were investigated. Two had greatly decreased duodenal pancreatic enzyme secretion in response to pancreozymin and secretin stimulation (one had inflammatory bowel disease and one had cystic fibrosis), and two had undergone pancreatectomy and had almost undetectable faecal chymotrypsin. The remaining child had cystic fibrosis and low faecal chymotrypsin concentrations, and was about to start pancreatic enzyme supplementation for poor weight gain. Their median (range) age was 2½ years (6 weeks–5 years) and weight 13.2 kg (4.8–17.4). Pancreatic enzyme supplements were discontinued 48 hours before the test.

Thirteen other subjects had normal pancreatic function. Nine had duodenal enzyme secretion within the reference range after stimulation by intravenous pancreozymin and secretin carried out during investigation of gastrointestinal symptoms. Four others with symptoms of chronic chest disease were under investigation for suspected cystic fibrosis, but subsequently shown to have normal sweat tests and faecal chymotrypsin concentrations. The median (range) age of the control subjects was 2 years (3 weeks to 13 years) and weight 9 kg (2.9–45.2).

After an overnight fast a urine specimen was taken before the test to determine the presence of interfering substances. Bentiromide 15 mg/kg body weight was then given, together with a standard breakfast of unmodified cows' milk and cornflakes. Babies not yet taking solids were given their usual milk feed. Urine was then collected for the next six hours. Adhesive collecting bags were used for children not old enough to cooperate with voiding. Older children were encouraged to drink fluids of their choice during the six hour collecting period and were allowed to eat lunch. The second phase of the test using free PABA alone (4.5 mg/kg) was

performed 48 hours later according to an identical protocol.

Urine volumes for the six hours were measured and aliquots of 20 ml frozen at -20°C until analysis. Urinary PABA was assayed by the Bratton and Marshall method for sulphanilamide as modified by Smith *et al.*⁸

MODIFIED ONE STAGE TEST, INCLUDING PAS

Twenty eight children with known pancreatic insufficiency were studied; 25 had cystic fibrosis proved by sweat testing, and had clinical steatorrhoea that had improved when enzyme supplements were given. The remaining three subjects, two with Shwachman's syndrome, and one who had had a pancreatectomy, had abnormally low pancreatic enzyme secretion in response to stimulation with pancreozymin and secretin. The median (range) age was 10 years (1 year 5 months–16 years) and weight 23 kg (8.2–54). Pancreatic enzyme replacements were stopped 48 hours before testing.

Twenty other subjects had normal pancreatic function. Fourteen had been investigated for diarrhoea with or without associated failure to thrive (five had enteropathies on histological examination of jejunal biopsy specimens) and had normal pancreozymin and secretin tests. There were two normal children, and four others who had normal stools but who failed to thrive from a variety of causes. Each of these had a negative sweat test that excluded cystic fibrosis, and their faecal chymotrypsin concentrations were within the normal range. Their median (range) age was 2½ years (4 months–11 years) and weight 8.5 kg (3–25).

The test protocol for the second group was similar to that for the first group except that 4.5 mg/kg PAS was given simultaneously with 15 mg/kg Bentiromide and a second day test was not performed. A 20 ml aliquot from the six hour urine collection was stored at -20°C until analysis.

After the alkaline hydrolysis of PABA and PAS conjugates, urine samples were assayed by high performance liquid chromatography as previously described⁹; the technique was modified to measure PABA and PAS simultaneously.⁷ In a few patients already receiving antibiotics, problems of interference with the measurement of PABA and PAS were overcome by using a 30×0.3 cm μ -Bondapak column (Millipore Ltd, Harrow, Middlesex) in place of the 15×0.5 cm polymer column previously described.⁹

Results from patients with pancreatic insufficiency were compared with those from subjects with pancreatic sufficiency by the Mann-Whitney U test. Because results for the control group in the six hour test were non-parametrically distributed, logarithmic

mic transformation of the data permitted calculation of the mean and two standard deviations as a reference range.

Approval for the study was obtained from the research ethical committee of the Central Birmingham Health Authority. Informed parental consent was obtained before the tests.

Results

The results of the two stage unmodified Bentriomide test are shown in fig 1. The median PABA excretion index for patients with exocrine pancreatic insufficiency was 29 (range 2–65) and for subjects with normal pancreatic function 60 (range 30–118, $p<0.05$) showing considerable overlap between the groups.

The results of the six hour PABA/PAS test are shown in fig 2. The PABA excretion index is derived from the ratio of concentrations of PABA and PAS derivatives in the urine.⁷ The median PABA excretion index for patients with pancreatic insufficiency was 19 (range 4–60) and for patients with pancreatic sufficiency 79 (range 66–140, $p<0.0001$) and there was complete separation between the two groups.

The PABA excretion index of the two patients with Shwachman's syndrome were 58 and 60, PABA recovery being 47% and 50%, respectively.

Fig 1 PABA excretion index results after unmodified two stage Bentriomide test of exocrine pancreatic function.

Fig 2 PABA excretion index results after modified one-stage Bentriomide test of exocrine pancreatic function, incorporating *p*-aminosalicylic acid.

Discussion

Results with the unmodified two day test (fig 1) show a wide spread of PABA excretion index in subjects with and without pancreatic insufficiency, with considerable overlap of the two groups. Though the number of patients studied with this test was small, the practical difficulties of a two day procedure and the wide spread of results suggest that it has little clinical application. In contrast, we have found the modified oral pancreatic function test using PAS as a pharmacokinetic marker for PABA a simple and accurate diagnostic test resulting in complete separation between patients with and without pancreatic exocrine insufficiency (fig 2). All patients with pancreatic sufficiency were correctly identified including those children with diarrhoea and failure to thrive secondary to an enteropathy. This makes the test particularly useful for rapid exclusion of pancreatic disease as a cause for altered bowel habit with or without failure of growth. The test also identified 93% of those subjects with pancreatic disease (the patients with Shwachman's syndrome being the two exceptions). Results with the modified test are therefore superior to recently reported findings using fluorescein dilaurate which, when given as an alternative to the

Bentiromide test, produced a false positive result in 20% of control subjects.¹⁰

Patients with the rare condition of isolated lipase deficiency cannot be identified by the Bentiromide test because of its chymotrypsin specificity, nor would an early indication of pancreatic exocrine failure such as decreased bicarbonate secretion be detected. In addition, the test cannot supplant conventional analysis of enzymes in aspirated duodenal juice for studying the ontogeny of pancreatic exocrine function, because it is likely that each pancreatic enzyme has a unique maturational pattern.¹¹

The two patients with Shwachman's syndrome, despite almost undetectable duodenal chymotrypsin secretion following pancreozymin and secretin stimulation, were still able to hydrolyse Bentiromide. Anomalous Bentiromide test results in patients with Shwachman's syndrome have been reported before,^{12 13} indicating that Bentiromide hydrolysis may not be entirely due to pancreatic chymotrypsin. Sterchi *et al* have characterised a small intestinal brush border enzyme capable of cleaving Bentiromide, and have named it PABA peptide hydrolase.¹⁴ There is a proximal to distal gradient in the activity of this enzyme with the lowest activity in the proximal small bowel. The activity of PABA peptide hydrolase was measured in jejunal biopsy specimens from both the children with Shwachman's syndrome. Interestingly, the child with negligible exocrine function showed the highest PABA peptide hydrolase activity (105.19 $\mu\text{mol}/\text{hour}/\text{gram}$ of protein; normal control mean (SD): 46.39 (6.97) $\mu\text{mol}/\text{hour}/\text{gram}$ of protein) whereas the second patient, who had some residual pancreatic function, had lower PABA peptide hydrolase activity (40.79 $\mu\text{mol}/\text{hour}/\text{gram}$ of protein), within the control range (M Lentze, personal communication). The importance of this finding remains uncertain. Shwachman's syndrome is a rare condition with a number of associated features,¹⁵ making it unlikely that a normal result from a Bentiromide test could in itself lead to misdiagnosis.

Despite these shortcomings the test has a number of clinical applications. It will readily exclude pancreatic exocrine insufficiency in most children being investigated for altered bowel habit, and would immediately call into question the diagnosis of cystic fibrosis if used in patients with a false positive sweat test.¹⁶ In addition, Bentiromide is useful in assessing the effectiveness of exogenous pancreatic enzyme replacement treatment in patients with proved pancreatic insufficiency.¹⁷

The PABA/PAS modification of the Bentiromide test overcomes a number of problems. A single collection of urine over six hours removes differ-

ences in completeness of urine collections in the two day test protocol that can be an important source of error. A quick and simple test is particularly useful in children who are not old enough to be able to void urine on demand. The test procedure itself proved acceptable to patients and no adverse reactions were associated with the administration of Bentiromide and PAS.

Using a colorimetric assay, up to 13% of tests fail because of interference from drugs and foodstuffs.¹⁸ Analysis by high performance liquid chromatography reduces this problem, though if patients are receiving antibiotics care must be taken to ensure that this does not interfere with analysis. Apparatus for high performance liquid chromatography is becoming standard in clinical chemistry laboratories, so that this test may be carried out in most district general hospitals. Alternatively, PABA and PAS conjugates remain stable in urine samples at ambient temperature for several days, and could be sent by post to a reference centre.

We recommend that when clinical features suggest pancreatic exocrine insufficiency, consideration should be given to using the modified PABA/PAS test before resorting to more invasive forms of investigation.

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