Preliminary evaluation of a single-day tubeless test of pancreatic function

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Abstract

The test for pancreatic exocrine function using *N*-benzoyl-L-tyrosyl-p-aminobenzoic acid (BTP test) does not require duodenal intubation, but misleadingly abnormal results often occur in patients with liver or bowel disease because the *p*-aminobenzoic acid (PABA) released by chymotrypsin hydrolysis of the peptide either is not conjugated or is malabsorbed. This study evaluated a modified BTP test, using a tracer dose of ^{14}C -PABA to eliminate misleading results, to assess exocrine function from a single six-hour collection of urine. The test clearly distinguished all patients with pancreatic steatorrhoea from normal subjects and identified patients with less severe pancreatitis as often as did the Lundh test. Furthermore, in patients with bowel or liver disease the misleadingly abnormal results of the unmodified BTP test were eliminated by the modified test in all but one case.

These findings suggest that the modified BTP test provides a practical alternative to conventional tests of pancreatic function that entail duodenal intubation.

Introduction

Standard methods of assessing pancreatic exocrine function entail duodenal intubation and analysis of aspirates after direct¹ or indirect² stimulation. An oral (tubeless) test of pancreatic function has been developed³ which uses the synthetic peptide *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BTP). Given by mouth, BTP is specifically hydrolysed by chymotrypsin⁴ to release *p*-aminobenzoic acid (PABA), which is absorbed and mostly conjugated in the liver before being excreted in urine. Measuring PABA and its metabolites in the urine provides an indirect index of pancreatic exocrine function.

Preliminary studies showed that the BTP test could detect pancreatic disease and that the results correlated well with tests using duodenal intubation.⁵⁻⁷ Nevertheless, misleadingly abnormal results often occur in patients with malabsorption or chronic liver disease because recovery of PABA in urine depends not only on chymotrypsin hydrolysis but also on the absorption and conjugation of PABA.⁸ Such misleading results can be excluded by performing a second test, replacing oral BTP by PABA, to correct for abnormal PABA absorption and metabolism.⁹

This two-day test is cumbersome for clinical use and we have therefore evaluated our suggestion for a single-day test using simultaneous BTP and a tracer dose of ^{14}C -PABA.⁹ We have applied the test also to patients with mild pancreatic damage and to patients with non-pancreatic steatorrhoea or liver disease, who are likely to have a misleading BTP test result.

Methods

The test (fig 1) was performed after an overnight fast. Since some foodstuffs (for example, apples and prunes) contain aryl acids, and some drugs (for example, sulphonamides, paracetamol, and frusemide) interfere with the laboratory analysis, a pretest urine sample was collected. A standard Lundh meal² containing 2 g BTP (Fluka AG, Basle) and 5 μCi (185 kBq) *p*-amino-[ring- ^{14}C]-benzoic acid (Radiochemical Centre, Amersham) was taken and the subjects instructed to drink plenty of fluids during the test. A six-hour urine

FIG 1—Single-day oral pancreatic function test. Test result calculated from chemical and isotopic recoveries of *p*-aminobenzoic acid (PABA).

sample was collected for analysis, enabling subjects to be tested as outpatients unless they were in hospital for other reasons. Urine samples were analysed for PABA content both chemically¹⁰ and isotopically (using liquid-scintillation counting). Each PABA recovery was calculated as the percentage of the oral dose excreted in the six-hour urine sample.

The specific activity of the ^{14}C -PABA used (7.2 mCi/mmol; 266 MBq/mmol) was such that the chemical dose it contained was negligible compared with the 680 mg of PABA in the BTP dose. As a result the chemical recovery of PABA in the urine is derived from BTP (dependent on chymotrypsin hydrolysis, absorption, and conjugation of PABA), whereas the isotopic recovery of PABA is derived from the ^{14}C -PABA (independent of chymotrypsin hydrolysis). The ratio of the two recoveries allows a correction to be made for PABA absorption or conjugation:

$$\text{PABA excretion index} = \frac{\text{6-h urinary PABA recovery from BTP}}{\text{6-h urinary } ^{14}\text{C recovery from } ^{14}\text{C-PABA}}$$

Lundh tests were performed by a standard method² and mean tryptic activity measured by the method of Wiggins.¹¹ Serum immunoreactive trypsin was measured using a commercial kit (RIAgnost, Hoechst).

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Subjects

We studied 51 subjects. All had given informed consent to the procedure, which was approved by the DHSS Administration of Radioactive Substances Advisory Committee and the local ethical committee.

Normal subjects comprised 10 healthy male medical staff aged 25-43 years who had no evidence of pancreatic disease and normal serum amylase and immunoreactive trypsin activities.

Pancreatic steatorrhoea—This group comprised nine patients (seven men) aged 28-64 years with a grossly abnormal Lundh test result and steatorrhoea that responded to pancreatic supplements. The diagnosis was supported by abnormal findings by at least one of retrograde cholangiopancreatography (five patients), ultrasonography (five patients), laparotomy (four patients), arteriography (one patient), or pancreatic calcification (one patient). Pancreatic insufficiency was caused by subtotal resection for carcinoma two and six years before in two patients and by alcohol abuse in one, and due to idiopathic chronic pancreatitis in the others.

Pancreatitis—Nine patients (seven women) aged 29-72 years had pancreatitis with no evidence of steatorrhoea. Three of these patients (all with an abnormal Lundh test result) had chronic pancreatitis confirmed by retrograde cholangiopancreatography (two patients) or laparotomy. The remaining six had a history of well-documented attacks of acute pancreatitis with hyperamylasaemia and abnormal appearances on ultrasonography, plus an abnormal CT scan or laparotomy findings in five patients. Two of these patients had persisting hyperamylasaemia (Lundh test abnormal) or pseudocyst formation after an acute attack, and the other four, with recurrent symptoms after acute pancreatitis six to 48 months before, had a normal Lundh test result at the time of the BTP test.

Bowel disease—Fifteen patients (11 men) aged 28-71 years had presented with steatorrhoea (eight patients), diarrhoea associated with weight loss or abdominal pain or both (five), or loss of weight alone (two). There was no clinical evidence of pancreatic disease in any of these patients. Lundh tests gave normal results in all 12 patients tested, who also had a normal pancreas at ultrasonography (five patients) or laparotomy (five); the underlying disease in these patients was postgastrectomy syndrome in four patients, irritable bowel syndrome in three, small-bowel resection for Crohn's disease or infarction in three, hereditary lymphangiectasia in one, and small-bowel contamination in one. The three patients who did not have a Lundh test had untreated coeliac disease, extensive Crohn's disease, and irritable bowel syndrome respectively; all responded well to appropriate treatment.

Liver disease—This group comprised five patients (three men) aged 37-56 years, who were jaundiced at the time of testing. Both a Lundh test and pancreatography gave normal results in the three patients tested. The final diagnosis in these patients was non-cirrhotic portal hypertension in two and alcoholic cirrhosis, chronic active hepatitis, and sclerosing cholangitis in the other three.

Mixed disease—Three patients with chronic pancreatitis, an abnormal Lundh test result, abnormal appearances on retrograde cholangiopancreatography, and steatorrhoea had coexistent disease (radiation ileitis, Crohn's disease, or primary biliary cirrhosis).

Results

Figure 2 shows the recoveries of PABA from BTP for the 51 subjects tested. In normal subjects the range of six-hour urinary PABA recovery was 51.5-70.0%. All patients with pancreatic steatorrhoea or mixed disease showed lower six-hour urinary PABA recoveries (12.5-45.5%); three patients with pancreatitis had a normal recovery—these patients also had a normal Lundh test result. Patients with bowel and liver disease showed a wide range of six-hour urinary PABA recoveries (range 15.0-67.1%); seven of the 15 with bowel disease and four of the five with liver disease had an abnormal result despite having no evidence of pancreatic disease.

The recoveries of ^{14}C from labelled PABA (reflecting its absorption and conjugation but independent of pancreatic function) were as expected. Normal subjects had slightly greater recoveries (66-85%) than from BTP. In the patients with pancreatic disease recovery of ^{14}C was up to four times greater (though sometimes still less than in controls) than the recovery from BTP. In patients with liver or bowel disease the wide range of ^{14}C recoveries (23-71%) was similar to those obtained from BTP.

When the correction for abnormal PABA metabolism was made the separation between groups was greatly improved (fig 3). All normal subjects had a PABA excretion index between 0.73 and 0.88,

which was clearly distinct from all nine patients with pancreatic steatorrhoea (excretion index 0.27-0.68) and mixed disease (0.37-0.61). Of the patients with pancreatitis, only four had an abnormal PABA excretion index; but four of the five patients with a normal index also had a normal Lundh test result, so that one patient with chronic pancreatitis, a grossly abnormal Lundh test result, and a cyst in the tail of the pancreas seen at retrograde cholangiopancreatography had a

FIG 2—Six-hour urinary PABA recoveries from oral dose of BTP. Considerable overlap occurred in pancreatic and non-pancreatic disease groups. Open circles denote normal Lundh test result. Closed triangles denote steatorrhoea.

FIG 3—Results of PABA excretion index in patient groups. Only one patient with non-pancreatic disease had abnormal result, and only one patient with pancreatitis and abnormal Lundh test had normal result. Open circles denote normal Lundh test result. Closed triangles denote steatorrhoea.

normal index. In the patients with liver or bowel disease the index was normal in all but one.

The modified, single-day test therefore almost eliminated the misleadingly abnormal results due to malabsorption or impaired conjugation of PABA, so that an abnormal PABA excretion index indicated pancreatic disease.

Discussion

In over 1200 subjects^{5-8 12-17} the unmodified BTP test reliably distinguished normal subjects from patients with chronic pancreatitis, though most of the latter had severe exocrine deficiency. Nevertheless, misleadingly abnormal results occur in the presence of other gastrointestinal or hepato-

biliary disease in 21% and 15% of patients respectively and are more common if steatorrhoea or cirrhosis is present even though exocrine function is normal.^{8 12 16} Such misleading results are unacceptable for diagnostic purposes and do not occur with tests using duodenal intubation.

This test requires only a single six-hour collection of urine and confirms our finding with the two-day test that modification to correct for abnormal PABA metabolism eliminates the misleading results in liver or bowel disease.⁹ This is important because the detection or exclusion of pancreatic disease in patients who present with symptoms that could be caused by either pancreatic or intestinal disease is a frequent clinical problem. The unmodified BTP test gives an abnormal result only in the presence of severe chronic liver disease, and patients presenting with jaundice are more appropriately investigated by other means. It is encouraging that chronic pancreatitis was indicated in the three patients with coexistent small-bowel and liver disease and that results were as good as with the Lundh test in all but one patient with less severe pancreatitis.

The present test does entail using an isotope, ¹⁴C-PABA, but the radiation dose is extremely small. The whole-body dose was estimated at less than 1 mrad (10 µGy) and the organ of reference, which was considered to be the small intestine, receives an estimated dose of 2.5 mrad (25 µGy). We used a high dose of BTP in this study, but other work¹⁸ suggests that a 500 mg dose given with 25 g casein improves the separation between groups.

Tetlow *et al*¹⁸ also used this single-day test, and their findings in normal volunteers and patients with chronic pancreatitis were similar to ours. Furthermore, out of 10 patients with non-pancreatic abdominal pain, three had an abnormal BTP test result (grossly so in one patient) but a normal result using the modified test; we thought that patients with non-pancreatic steatorrhoea provided a more rigorous test of the modification. Two other tubeless tests have been advocated, both of which entail longer urine collection times (10-24 hours). The fluorescein dilaurate test¹⁹ also takes two days to perform, and hydrolysis of the diester by pancreatic enzymes may not be specific and is bile dependent. The dual-label Schilling test²⁰ gave good preliminary results but isotope recoveries were low.

An advantage of the single-day modified BTP test, which essentially measures digestive ability, is its relevance to the clinical picture in steatorrhoea. Nevertheless, 90% of the pancreas has to be destroyed before steatorrhoea occurs,²¹ so that theoretically the test should be less sensitive than tests using duodenal intubation; but in practice the tubeless test has also detected most patients with exocrine insufficiency but no steatorrhoea.

Preliminary findings with the test suggest that it may prove useful in detecting pancreatic disease. It is potentially as accurate as conventional tests and avoids duodenal intubation. The practical benefits are a saving in time, facilities, and skill for the clinician and lack of discomfort for patients. Furthermore, the failure rate with tests using duodenal intubation is about 10% even in the most expert hands.

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FLUX-WEED rises up with a round upright hard stalk, four or five feet high, spread into sundry branches, whereon grow many greyish green leaves, very finely cut and severed into a number of short and almost round parts. The flowers are very small and yellow, growing spike fashion, after which come small long pods, with small yellowish seed in them. The root is long and woody, perishing every year.

There is another sort, differing in nothing, save only it has somewhat broad leaves; they have a strong evil savour, being smelled unto, and are of a drying taste.

They flower wild in the fields by hedge-sides and highways, and among rubbish and other places. They flower and seed quickly after, namely in June and July.

This herb is saturnine also. Both the herb and seed of Flux-weed is of excellent use to stay the flux or lask of the belly, being drank in water wherein gads of steel heated have been often quenched; and is no less effectual for the same purpose than Plantain or Comfrey, and to restrain any other flux of blood in man or woman, as also to consolodate bones broken or out of joint. The juice thereof drank in wine, or the decoction of the herb drank, doth kill the worms in the stomach or belly, or the worms that grow in putrid and filthy ulcers, and made into a salve doth quickly heal all old sores, how foul or malignant soever they be. The distilled water of the herb works the same effect, although somewhat weaker, yet it is a fair medicine, and more acceptable to be taken. It is called Flux-weed because it cures the flux, and for its uniting broken bones, &c. Paracelsus extol it to the skies. It is fitting that syrup, ointment, and plaisters of it were kept in your house. (Nicholas Culpeper (1616-54) *The Complete Herbal*, 1850.)