Exocrine Pancreatic Function as Determined in a Same-Day Test with Use of Bentiromide and p-Aminosalicylic Acid

Jonathan D. Berg,¹ Ian M. Chesner,² Rosalind A. C. Allen-Narker,¹ Brendan M. Buckley,¹ and Nigel Lawson³

We describe a new approach to the bentiromide test of exocrine pancreatic function. p-Aminosalicylic acid (PAS), a compound closely related to the bentiromide fragment p-aminobenzoic acid (PABA), is used as a marker of the pharmacokinetic behavior of PABA to derive a PABA excretion index. This index is identical to that derived with [14C]-PABA. Concentrations of both PABA and PAS are measured in urine by "high-performance" liquid chromatography, which avoids the drug interferences encountered with established assays of PABA. We discuss the practical and diagnostic advantages of this new approach to the bentiromide test.

Additional Keyphrases: p-aminobenzoic acid (PABA) • chromatography, liquid • electrochemical detection

The bentiromide test, widely used in the investigation of exocrine pancreatic function (1–3), is based on the specific hydrolysis, catalyzed by pancreatic chymotrypsin (EC 3.4.21.1), of orally administered bentiromide (N-benzoyl-L-tyrosyl-p-aminobenzoic acid) in the gut. The p-aminobenzoic acid (PABA) that is released is absorbed and may be measured in either urine or serum.

Two main factors, analytical interference and pharmacokinetic variation, have limited the usefulness of the test. The colorimetric assay for measuring PABA (4) lacks specificity, being susceptible to interference by several common drugs and components of foodstuffs (5,6), which may lead to erroneous results or to test failure. In a recent clinical study, the test had a 13.7% failure rate owing to drug interference with PABA analysis (7). Such interference can be avoided if PABA is measured by "high-performance" liquid chromatography (5,9).

Abnormalities in the pharmacokinetics of PABA—from alterations in gastrointestinal absorption, hepatic function, and renal excretion—also interfere with the interpretation of test results. For example, patients with steatorrhea investigated for pancreatic exocrine insufficiency may instead have impaired intestinal function, which decreases PABA absorption and gives a false-positive result. This problem has been approached in the past either by administering PABA on a separate day from bentiromide (10) or by concurrent administration of bentiromide and [14C]PABA (11,12). The ratio of bentiromide-derived PABA to control is usually expressed as a PABA excretion index. However, these approaches are inconvenient to patients and hospital staff or involve exposure to radioisotope.

Here we describe a new one-day test involving p-aminosalicylic acid (PAS) as a pharmacokinetic marker to determine a PABA excretion index.

Structurally and pharmacokinetically related to PABA, PAS has a long-established record of safe therapeutic administration. Its concentrations in urine can conveniently be measured simultaneously with PABA by liquid chromatography.

Materials and Methods

Subjects: The study protocol was approved by the Ethics Committees at both participating institutions. Twenty healthy volunteers (10 men and 10 women, ages 20 to 55 years) participated in establishing normal reference intervals for the new test. We have previously described (9) a reference range for an excretion index based on [14C]PABA in healthy volunteers, as determined with liquid chromatography.

We also tested six patients who had exocrine pancreatic insufficiency, diagnosed on the basis of steatorrhea, with fecal fat excretion ranging from 35 to 110 mmol/day and abnormal secretin/pancreozymin tests with low activities of lipase (EC 3.1.1.3) and trypsin (EC 3.4.21.4) and low bicarbonate secretion after stimulation. Six other patients with various gastrointestinal abnormalities (Table 1) were also screened for pancreatic dysfunction. In this second group of patients, confirmative tests of pancreatic function had not been undertaken.

Test protocol: Subjects discontinued taking pancreatic supplements 48 h before the start of the test. On the morning of the test, after collecting a pre-test urine specimen, the subjects each took 1 g of bentiromide and 300 mg of PAS (Roche Products Ltd., Welwyn Garden City, Hertfordshire, U.K.) with 25 g of casein in 400 mL of chocolate-flavored drink. Patients but not healthy volunteers also received  μCi of [carboxy-14C]PABA (Amersham International, Amersham, Bucks., U.K.), as previously described (9). Control subjects did not receive [14C]PABA. To obtain adequate diuresis the subjects drank 1 L of water during the test. All urine produced for 6 h after the start of the test was collected.

Assay of PABA and PAS: After the alkaline hydrolysis of PABA and PAS conjugates, we assayed urine samples by liquid chromatography as previously described (9), with the following modifications for measuring PAS simultaneously with PABA. The hydrolysis partially decarboxylates PAS to 3-aminophenol, which is also measured with PAS and PABA in the chromatographic system. We prepared aqueous standards of PAS (2 mmol/L), PAS (2 mmol/L), and 3-aminophenol (0.5 mmol/L). Standards and urine samples were diluted in 8 ml/L NaOH containing 0.5 mol of m-hydroxybenzoic acid per liter as an internal standard.

Except for the PAS standard, we hydrolyzed all samples at 120 °C for 1 h, then diluted 10 μL of the hydrolysates 100-fold in mobile phase (pH 2.6). The PAS standard was diluted just after we added it to the NaOH, to avoid any decomposition to 3-aminophenol. After the mixture was centrifuged to remove particulate matter, we injected 20 μL of the diluted hydrolysates onto the chromatographic system.

---

¹ Department of Clinical Biochemistry, Sandwell District General Hospital, West Bromwich, B71 4HJ, U.K.
² The Metabolic Unit and ³ the Department of Clinical Chemistry, East Birmingham Hospital, Birmingham, B9 5ST, U.K.

Received February 3, 1986; accepted March 12, 1986.
We used a 150 × 4.6 mm column of PLRP-S (8-μm particle size) polymer (Polymer Laboratories, Church Stretton, Shropshire, U.K.). The mobile phase was acetonitrile/water (1/7 by vol), pH 4.3, containing 100 mmol of sodium dihydrogen phosphate per liter. The flow rate was 1 mL/min. PAS, PABA, and 3-aminophenol were detected electrochemically at an oxidation potential of 1.1 V and sensitivity of 300 nA with a Model LCA15 electrochemical detector (EIT Research, London, U.K.).

Using urine samples to which analyte was added, we determined that the sum of the concentrations of PAS and 3-aminophenol yields a recovery of between 97 and 101% of the original amount of PAS present. No other breakdown products are detectable.

**Results**

PABA, PAS, and 3-aminophenol were easily resolved, as illustrated by Figure 1a, which shows a chromatogram of urine collected from a healthy volunteer during the 6 h of the test. In this subject the 6-h excretion of PABA was 75% of the dose taken and that of PAS (PAS plus 3-aminophenol) 78%, giving a PABA excretion index (PABA/PAS) of 96.

Pre-test urine samples from patients or volunteers contained no material with the chromatographic characteristics of PABA, PAS, or 3-aminophenol. Figure 1b shows a chromatogram of pre-test urine from a patient with chronic pancreatitis. The 6-h urine specimen from the same patient (Figure 1c) yielded a small PABA peak corresponding to a 6-h PABA excretion of 5% of dose taken. In this patient the excretion of PAS during 6 h, measured as PAS plus 3-aminophenol, was 79%, giving a PABA excretion index of 6.

Table 1 presents data on PABA, PAS, and [14C]PABA in 6-h urine specimens from healthy individuals and patients. In controls, the PABA excretion index derived by using PAS was very close in both mean and range to that which we previously found by using [14C]PABA. Furthermore, in patients no significant differences were found between the two PABA excretion indexes, irrespective of whether pancreatic or gastrointestinal dysfunction was present. Comparison of both excretion indexes in the 12 patients by Deming regression analysis shows that they are highly correlated: the regression equation was $y = 1.006x - 0.842$ ($r = 0.986; p < 0.001$), where $y$ is the PAS-derived and $x$ is the [14C]PABA-derived index. Patients with proven chronic pancreatitis were easily distinguished from controls by either index in all cases ($p < 0.0001$ for both indexes).

**Discussion**

The PABA excretion index increases the specificity of the bentiromide test (5, 12) by correcting for pharmacokinetic variation. However, administration of free PABA requires a second test day and neglects day-to-day variation in PABA kinetics. Although these problems are avoided when [14C]PABA is used, its administration to certain patients—e.g., children—is undesirable, and it entails an additional analytical procedure.

The pharmacokinetic behavior of PAS is very similar to that of PABA because of the close structural similarity of the two compounds (13). Until relatively recently, PAS was in widespread use as an antitubercular agent and its safety is established. The 300-mg dose of PAS taken in this test is low as compared with daily doses of between 10 and 20 g used in the treatment of tuberculosis. Other compounds structurally similar to PABA, for instance PABA isomers,
potentially might be used as pharmacokinetic markers. However, unlike PAS, their pharmacology and safety would need extensive investigation before they could be used as part of a diagnostic test.

The results we report show that the PABA excretion index derived by using PAS is close to that obtained by using $^{14}$CPABA, reflecting the similarity in pharmacokinetic behavior of PAS and PABA. The present assay is much more specific than colorimetric methods and also enables simultaneous measurement of PABA and PAS.

Use of combined preparations containing a fixed ratio of bentiromide to PAS would avoid the need for precise calculation of dose based on body weight and would greatly increase the convenience of the test for use in children where the use of radioactivity is undesirable (14).

The novel procedure we describe here facilitates interpretation of the bentiromide test and should help extend its use in the investigation of pancreatic exocrine dysfunction.

We thank Drs. A. J. Shipman and K. A. Cleur of Roche Products and Jane E. Humphreys of Clinical Project Services for their support.

References