Changes in Alkaline Phosphatase and 5’-Nucleotidase Multiple Forms After Surgical Management of Biliary Obstruction

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Serial measurements of alkaline phosphatase and 5’-nucleotidase multiple forms in two patients undergoing surgical procedures to release biliary obstruction suggested an inverse relationship between high-M₄ isoforms and serum bile acids concentrations. Furthermore, the study of several groups of patients with cholestatic disorders confirmed this inverse correlation. Mechanisms responsible for these observations are discussed.

Additional Keyphrases: cholestasis • bile acids

Measurement of serum bile acids (SBA) is a useful tool for assessing hepatic cholestatic damage. Bile acids are known to increase hepatic synthesis of alkaline phosphatase (ALP; EC 3.1.3.1) (1) and possess detergent properties.

High-M₄ enzymatic forms appear in the serum of patients with cholestasis. Shedding of hepatocyte membrane fragments containing enzymes associated with liver plasma membrane seems to be a likely origin of the high-M₄ (biliary) isoform of ALP (HM₄-ALP) and the isoform of 5’-nucleotidase with a1 electrophoretic mobility (IsoNuc1) (2–4).

HM₄-ALP is generally accepted as a good indicator of cholestasis (5) and has been advocated as a useful marker of liver metastases (6). Detergents are known to convert this isoform in the hepatic form of ALP (7). We recently reported (4) that the IsoNuc1 of 5’-nucleotidase (5’-NU; EC 3.1.3.5) is significantly increased in cholestatic patients having high amounts of HM₄-ALP, with the ratio of total 5’-NU to β 5’-NU being consequently increased. Treatment of samples with Triton X-100 transforms the IsoNuc1 in the fraction with β mobility, resulting in a decreased ratio (4).

Here we studied SBA concentrations and ALP and 5’-NU multiple forms in two patients undergoing surgical procedures to release biliary obstruction, as well as in several groups of patients with different cholestatic disorders.

Materials and Methods

We assayed total SBA at 37 °C by an enzymatic colorimetric reaction (Merck, no. 14352, Darmstadt, FRG) with a Cobas-Fara II centrifugal analyzer (Hoffmann-La Roche Co., Ltd., Basel, Switzerland) (8). We assayed serum 5’-NU activity at 30 °C by Enzymel 5NU Optimised reagent kit (BioMérieux, Charbonnières les Bains, France) with a Cobas-Fara II centrifugal analyzer. We assayed serum ALP activity at 30 °C in a Hitachi 737 autoanalyzer with reagents based on the method recommended by the German Society of Clinical Chemistry (Boehringer Mannheim, no. 1 040 669, Mannheim, FRG).

We electrophoretically separated ALP multiple forms on cellulose acetate plates by using the kit supplied by Helena France SA, which includes treatment of the samples with neuraminidase to separate liver and bone isoforms. We separated serum 5’-NU multiple forms on cellulose acetate electrophoresis plates (Titan III IsoFlur, Helena Labs., Beaumont, TX), as described elsewhere (4).

Because most variables did not follow a normal distribution according to skewness and kurtosis coefficients, we used nonparametric statistical methods in calculations and median and semi-interquartile range as descriptive indexes.

Our study included 80 patients with hepatobiliary cholestatic disorders. They were divided into seven etiologic groups according to clinical, imaging, and pathological criteria: Cir, 24 patients with liver cirrhosis; Hep, 6 patients with acute hepatitis; M/HC, 9 patients with liver metastases and (or) hepatocarcinoma; Int, 10 patients with intrahepatic processes (lymphoma, sepsis, a1-antitrypsin deficiency, pulmonary thromboembolism); UGH, 8 patients with liver cirrhosis and upper gastrectestinal hemorrhage; CHP, 7 patients with cancer of the head of the pancreas; and NBT, 16 patients with neoplasia of extrahepatic biliary tract.

We obtained serial samples from two patients undergoing surgical procedures to relieve biliary obstruction. Patient 1 had an ampulloma with complete obstruction to bile flow that was released surgically. Patient 2 had a liver neoplasia with complete bile duct obstruction that was released surgically. Samples before and after surgery were obtained from both patients.

Results

Table 1 shows the values of the different variables in four samples from patient 1 (three samples before sur-
surgery and one sample 8 days postsurgery) and in two samples obtained from patient 2 (one sample before and one sample 12 days after surgery). In both patients a marked inverse relationship is observed between HM-ALP and SBA, and IsoNuc1 and SBA. Before surgery, SBA concentrations were high, HM-ALP was around or below the detection limit of the technique, and IsoNuc1 and the total ALP/β 5'-NU ratio were not elevated. After cholestasis was surgically released, SBA returned to a normal concentration, but HM-ALP, IsoNuc1, and the ratio were all dramatically increased.

Table 2 shows the median and the semi-inter quartile range of variables for the different groups of cholestatic patients. SBA are significantly lower in patients with hepatoma and (or) liver metastases than in patients with cirrhosis (P <0.01), carcinoma of the head of the pancreas (P <0.001), and neoplasia of the biliary tree (P <0.01). Inversely, HM-ALP and IsoNuc1 are significantly higher in patients with hepatoma and (or) metastases than in patients with cirrhosis (P <0.001) and carcinoma of pancreatic head (P <0.01 for IsoNuc1, P <0.05 for HM-ALP).

Table 3 shows Spearman correlation coefficients between these variables in all cholestatic patients. Both IsoNuc1 and the total 5'-NU/β 5'-NU ratio correlate well with HM-ALP, and the correlation between IsoNuc1 and the ratio is also satisfactory, as previously described (4). Surprisingly, SBA concentrations show significant negative correlation coefficients with HM-ALP, IsoNuc1, and the total 5'-NU/β 5'-NU ratio.

Discussion

The inverse relationship between high-M₇ enzymatic forms and SBA concentrations in patients with cholestasis is rather surprising, because increasing values for all these variables is expected with the progression of biliary obstruction. Furthermore, it is not easy to understand why concentrations of these enzymatic forms increase in serum after surgery has released the biliary obstruction.

Several hypotheses could explain our findings. Surgical management of biliary obstruction makes SBA concentrations decrease, whereas simultaneous shedding of membrane-associated enzymes into blood could result from the surgical handling of the biliary tree. However, the decrease of total ALP and 5'-NU activities after surgery argue against this. In vivo conversion of high-M₇ enzymatic complexes to lower-M₇ soluble forms is another possible mechanism. Detergents such as Triton X-100 are known to transform HM-ALP in the hepatic isoform of ALP (7). Also, IsoNuc1 is converted to the fraction with β mobility by Triton X-100 (4). Long-standing high concentrations of SBA could have a similar effect in vivo, and increased concentrations of serum deoxycholic acid may produce false-negative results when HM-ALP is used as a marker of liver metastases (9).

Although further experimental work is needed to confirm possible interactions between SBA and High-M₇ enzymatic forms, our results indicate that electrophoretic separations of ALP and 5'-NU multiple forms should be interpreted cautiously, especially when they are included in the diagnostic workup of a patient with suspected liver metastases or obstructive biliary processes of tumoral origin.
References


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Measuring Albumin and Calcium in Serum in a Dual Test with the Hitachi 704

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We describe a method for simultaneously determining albumin, by using brom cresol purple, and calcium, by using Arsenazo III, in the same analytical cuvette on the Hitachi 704. Both assays agree well with accepted procedures. The standard curves for the albumin and calcium assays are linear from 0 to 60 g/L and 0 to 5.0 mmol/L, respectively. Calibration is stable for 7 days with use of open reagent in the instrument. Both assays are unaffected by hemoglobin ≤5 g/L and Intralipid ≤4 g/L; calcium is unaffected by bilirubin ≤600 μmol/L.

Additional Keyphrases: colorimetry • multianalyte assay

Recent developments in automated spectrophotometric instrumentation include the ability to simultaneously measure absorbances at several different wavelengths several times a minute. Many of the large analyzers currently available operate two-reagent chemistries with the same throughput as single-reagent chemistries; this has prompted the development of dual assays in which reagent 1 initiates one analysis and reagent 2 another. This approach has three main advantages: increasing the test throughput, broadening the test range on the instrument by making an extra channel available for every assay pairing, and reducing the total sample volume required. Already Boehringer Mannheim (Mannheim, FRG) offers combination assays of urea/glucose, cholesterol/triglycerides, and alanine/aspartate aminotransferases.

Albumin and calcium concentrations are frequently requested clinical analytes. Because ionized calcium is the metabolically active form and albumin is the principal ligand for calcium in plasma, the requests form a natural pair.

Albumin determinations by dye binding are carried out at pH 5.5 for brom cresol purple (BCP) (1) and pH 4.4 for brom cresol green (2). However, the widely used cresolphthalein complexone estimation for calcium is performed at pH 11.0 (3). Recently, Arsenazo III has been used as a complexing agent for measuring plasma calcium (4). This assay is usually carried out around pH 6.0, opening up the possibility of first measuring albumin with BCP at 600 nm, followed by measuring calcium with Arsenazo III at 660 nm. Here we describe conditions whereby both albumin and calcium can be sequentially measured in the same cuvette with the Hitachi 704.

Materials and Methods

Materials

PIPS, Arsenazo III, and bilirubin (from gallstones) were obtained from Sigma Chemical Co. (St. Louis, MO). Intralipid was supplied by Kabivitrum (Cahill May Roberts, Dublin, Ireland). Antiserum to albumin was supplied by Dako (Glæstrup, Denmark). All other chemicals were Analar grade from British Drug House Ltd. (Poole, UK).

BCP reagent consisted of 50 mmol/L acetate buffer, pH 5.5, containing BCP, 120 μmol/L, and Brij 35 (30%) surfactant, 1 mL/L.

Arsenazo III reagent consisted of 1,4-piperazine diethanesulfonic acid buffer (50 mmol/L, pH 6.8) containing 500 μmol of Arsenazo III per liter.

Methods

We used a Hitachi (Tokyo, Japan) Model 704 automated analyzer. Given that a sample volume fraction of ≤0.01 is necessary for good linearity in the BCP assay and given the desirability of using the maximum sam-

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